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American Journal of Diseases of Children

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BLOOD STUDIES IN THE NEW-BORN

MORPHOLOGICAL; CHEMICAL; COAGULATION; UROBILIN AND BILIRUBIN *

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BRADFORD FRENCH DEARING, M.D.; HAL R. HOOBLER, M.D.;
ANITA COX; MARTHA R. JONES, PH.D.,
AND
FRANCIS SCOTT SMYTH, M.A.
SAN FRANCISCO

INTRODUCTION

Reviewing the literature on blood studies one is struck by the comparatively few extensive investigations which have been carried on with the blood of the new-born. The morpohologic variations in hemoglobin and cells have received more consideration than any other phase of the problem. The chemistry of the blood of the new-born has been given little attention. Sedgwick's [1] findings stand out as the most extensive and illuminating, and Slemon's [2] investigations on maternal and fetal blood are fundamental, furnishing conclusive evidence of the mechanistic function of the placenta. Coagulation has been given little real attention and except for isolated studies on individual cases of hemorrhages of the new-born few, beside Rodda,[3] have attempted to analyze the factors entering into this problem.

We have, therefore, attempted to bring together all these phases—morphology, chemistry, coagulation and pigment metabolism—into a combined study of the blood of the normal new-born infant. All blood

* From the Hooper Foundation of Medical Research.

* This work was made possible in part by a grant from Mr. William H. Crocker.

* From the Department of Pediatrics, University of California Medical School. This paper represents Series No. 6, Studies on Blood, which includes the following papers: 1. A Study of the Blood in Hemophilia, S. H. Hurwitz and W. P. Lucas. 2. Blood Volume in Infants Estimated by the Vital Dye Method, William Palmer Lucas and Bradford French Dearing. 3. Distribution of Calcium in Phosphoric Acid in the Blood of Normal Children, Martha R. Jones and Lillian L. Nye. 4. On the Etiology of Hemorrhagic Disease of the New-Born, Clain Fanning Gelston. 5. Physiology of the Blood in Infancy and Childhood, W. P. Lucas.

1. Sedgwick: Am. J. Dis. Child. **14**:98 (Aug.) 1917; **19**:429 (June) 1920.
2. Slemon: Bull. Johns Hopkins Hosp. **27**:343 (Dec.) 1916; J. Biol. Chem. **32**:63 (Oct.) 1917; Am. J. Obst. **80**:194 (Aug.) 1919.
3. Rodda: Am. J. Dis. Child. **19**:269 (April) 1920.

specimens were obtained from longitudinal sinus puncture, the blood being obtained at intervals from a few hours after birth to the fourteenth day post partem. In a few cases later specimens were obtained, if the child remained in the hospital for a longer period, but few of these findings are presented, except as they represent a continuation of a serial study on the identical baby. Most of our figures represent serial studies; i. e., blood obtained on alternate days from the same baby. In this way we have studied over one hundred and fifty babies. It would be impossible, of course, to make all the determinations we wished from the amount of blood we felt it safe to take from each baby, so that the numbers in the various series differ considerably, but in each case we think a sufficient number of examinations were made to show the variations that occur normally in the blood of the new-born infant.

HEMOGLOBIN

The estimation of hemoglobin by most of the clinical methods now in vogue, as has been pointed out frequently during the past few years, is very faulty and inaccurate, giving errors as high as 20 or 30 per cent. and in some instances as high as 50 per cent. as compared with the methods of estimating the hemoglobin by the oxygen capacity of the red blood cells either by the Palmer [4] method or by the modification of this method as described by Robscheit.[5] From clinical experience we have found this method (acid hematin) very satisfactory and reliable. Our tables for hemoglobin show the same high values found by most workers during the first few days with a decided drop during the second week. This bears out the estimations made by Williamson of the total hemoglobin in grams per hundred c.c. of blood.

Williamson [6] has accurately determined the amount of hemoglobin in grams per hundred c.c. of blood in infants, children and adults. He found that on the first day there are 23.25 gm.; for the second and third days, 22.78 gm.; fourth to eighth days, 22.12 gm.; ninth to thirteenth days, 21.35 gm. The decrease in hemoglobin continues to the first year and second year when it is 12.53 and 12.57 gm., respectively. From that time on it gradually increases till it reaches its fairly constant average between the sixteenth and twentieth years of 16.25 gm. hemoglobin per hundred c.c. of blood. There is a very decided drop from 21.35 to 18.42 gm. between the thirteenth day and the period from two weeks to two months. In the third to fifth months it drops to 13.66 gm. and from the sixth to the eleventh months it remains about the

4. Palmer: J. Biol. Chem. **41**:209, 1920.
5. Robscheit: J. Biol. Chem. **41**:209, 1920.
6. Williamson: Arch. Int. Med. **18**:505 (Oct.) 1916.

same, 13.70 gm. Williamson concludes that the amount of hemoglobin in the blood of normal persons varies greatly at different ages and follows a well defined curve. The variations are greatest from birth to sixteen years.

Our results on hemoglobin estimation are shown in Table 1.

This average corresponds very closely with the average results of E. Schiff [7] which were made at Prague and Budapest.

TABLE 1.—RESULTS ON HEMOGLOBIN ESTIMATION

Days	Cases	Maximum	Average	Minimum
1	36	140	117	85
2	27	135	114	100
3	17	135	110	80
4	26	130	114	95
5	18	125	107	75
6	21	130	113	80
7	16	125	109	75
8	20	125	103	60
9	17	115	103	90
10	13	120	97	70
11	18	120	98	70
12	12	115	91	55

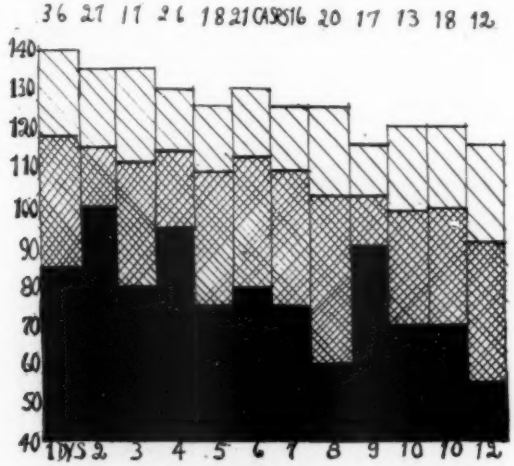

Fig. 1.—Hemoglobin from sinus blood.

Williamson [8] reports his studies on grams of hemoglobin per hundred c.c. of blood. His average finding on the first day was 23.25 gm., this gradually decreasing in amount until the age of two years was reached (12.57 gm.). From this age to fifteen years there was a gradual increase to 14.69 gm. Our findings on the hemoglobin per cent. in the first few weeks of life show a curve parallel to his curve.

7. Schiff, E.; von Reuss, August Ritter: The Diseases of the New-Born, New York, William Wood & Co., 1921.

8. Williamson: Arch. Int. Med. **18**:521 (Oct.) 1916.

Hiemann [9] states that the hemoglobin of jaundiced infants is distinctly lower than that of normal infants. We have not been able to demonstrate this as the hemoglobin in some of our most markedly jaundiced infants was just as high as in infants without jaundice. As we shall show, bile pigment is present in nearly all new-born infants even though they are clinically free from icterus. As our estimates were made from blood obtained from the longitudinal sinus, we compared these findings with a series in which the blood was taken from puncture in the usual way. Our tables show slight variations, the sinus hemoglobin tending to be higher (Tables 2, 3 and 4).

TABLE 2.—HEMOGLOBIN OF SINUS AND PERIPHERAL BLOOD COMPARED

Days		Cases	Maximum	Average	Minimum
1.	Sinus	16	140	124	110
	Peripheral	13	145	130	120
2.	Sinus	16	135	122	105
	Peripheral	9	122	118	110
3.	Sinus	7	125	114	100
	Peripheral	8	...	115	98
4.	Sinus	17	130	119	108
	Peripheral	8	125	111	98
5.	Sinus	9	125	112	100
	Peripheral	7	118	109	100
6.	Sinus	18	130	108	90
	Peripheral	6	110	103	94
7.	Sinus	11	125	115	95
	Peripheral	5	108	101	95
8.	Sinus	11	125	108	100
	Peripheral	4	100	94	95

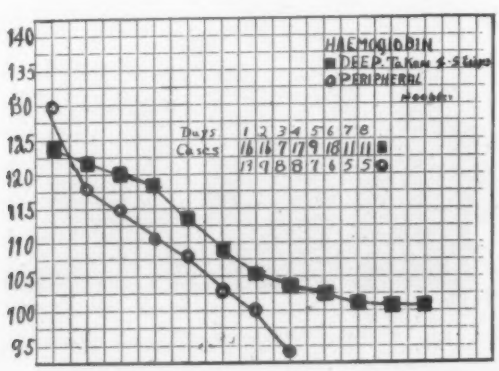

Fig. 2.—Comparing hemoglobin from sinus blood and peripheral blood.

RED BLOOD CELLS

The red blood cell counts are high during the first week and then gradually decrease, corresponding very closely with the hemoglobin estimations (Tables 3 and 4).

9. Hiemann: Zur Lehre des Ikterus Neonatorum, Ztschr. f. Geb. u. Gyn. **49**:165, 1911.

As is shown in the tables, the variations between the lowest counts and the highest are quite marked and correspond very well with the variations between the highest and lowest percentage of hemoglobin. The difference between the sinus and peripheral counts is slightly in

TABLE 3.—RED BLOOD CELL COUNTS FOR TWELVE DAYS

Days	Cases	Maximum	Average	Minimum
1	31	6,700,000	5,511,000	4,000,000
2	26	6,200,000	5,352,000	4,100,000
3	17	7,800,000	5,116,000	3,500,000
4	25	6,200,000	5,244,000	4,000,000
5	15	6,700,000	4,983,000	3,800,000
6	20	6,600,000	5,114,000	2,600,000
7	16	6,000,000	5,108,000	3,800,000
8	19	5,600,000	4,504,000	3,800,000
9	20	5,700,000	4,891,000	3,800,000
10	13	5,500,000	4,130,000	2,800,000
11	18	5,700,000	4,561,000	3,500,000
12	12	5,800,000	4,533,000	2,000,000

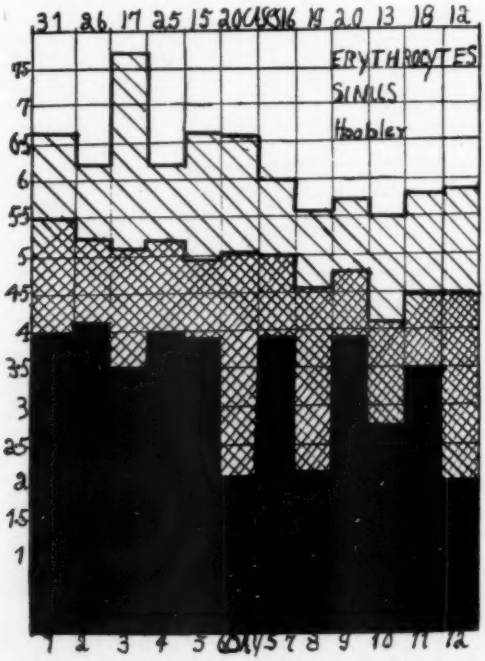

Fig. 3.—Red blood cells from sinus.

favor of the sinus counts, though this is not striking but it is interesting as it corresponds with the hemoglobin findings. Such variations in peripheral and deep circulation may depend on a number of different factors, as rate of circulation, volume, temperature, oxygen needs and exchange, etc.

Hiemann found values lower in icteric babies than in those free from icterus—5,500,000 as compared with 6,500,000. This we were unable to substantiate.

Much discussion has taken place on the values of the hemoglobin and red blood cells depending upon whether the cord is tied immediately or late, i.e., when pulsation ceases in the maternal half of the cord. According to Schiff, when the cord is ligated late the red blood cells rise between the second and fourth days, but when it is tied early the

TABLE 4.—RED BLOOD CELLS OF SINUS AND PERIPHERAL BLOOD

Days		Cases	Maximum	Average	Minimum
1.	Sinus	16	6,800,000	5,650,000	4,760,000
	Peripheral	12	7,024,000	6,080,000	5,336,000
2.	Sinus	15	5,952,000	5,295,000	4,192,000
	Peripheral	9	5,892,000	5,430,000	4,900,000
3.	Sinus	7	5,645,000	5,426,000	4,510,000
	Peripheral	8	7,000,000	5,238,000	4,432,000
4.	Sinus	17	6,108,000	5,268,000	4,162,000
	Peripheral	7	5,720,000	5,180,000	4,656,000
5.	Sinus	9	6,016,000	5,082,000	4,120,000
	Peripheral	7	5,072,000	4,962,000	4,464,000
6.	Sinus	16	6,624,000	5,063,000	3,936,000
	Peripheral	6	5,820,000	4,720,000	4,500,000
7.	Sinus	10	6,060,000	5,119,000	3,924,000
	Peripheral	5	4,950,000	4,900,000	4,550,000
8.	Sinus	11	5,248,000	4,800,000	4,320,000
	Peripheral	5	5,616,000	4,702,000	4,216,000

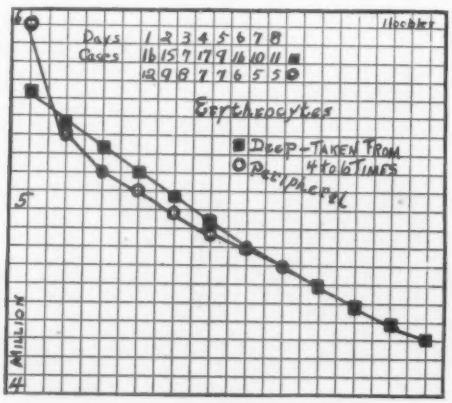

Fig. 4.—Comparison of red blood cells from sinus and peripheral circulation.

fall begins immediately. As all our cases had their cords tied late, i.e., when the pulsation stopped in the middle of the cord, it can be seen from our figures that the red blood cells have no definite tendency to rise between the second and fourth days, as claimed by Schiff.

There are certain definite things to be observed about the red blood cells of the new-born:

1. We found nucleated cells in 52 per cent. of all cases on the first day (1 per cent. of differential count) and in only 5 per cent. on the second day (0.5 per cent. of differential count).

2. Variation in size is more marked in the early days, larger and smaller cells both being more numerous (showing disturbance in equilibrium of the red blood cell producing organs).

TABLE 5.—WHITE CELLS FROM SINUS BLOOD

Days	Cases	Maximum	Average	Minimum
1	31	30,000	19,200	6,400
2	22	38,000	15,300	8,400
3	16	20,000	11,100	7,200
4	25	19,000	10,300	6,400
5	17	14,000	10,900	4,600
6	19	17,000	11,500	7,400
7	16	21,000	12,100	7,400
8	21	45,000	12,400	6,400
9	20	23,000	12,600	6,200
10	12	18,800	12,200	7,600
11	18	17,400	12,700	6,500
12	12	22,200	13,200	7,000

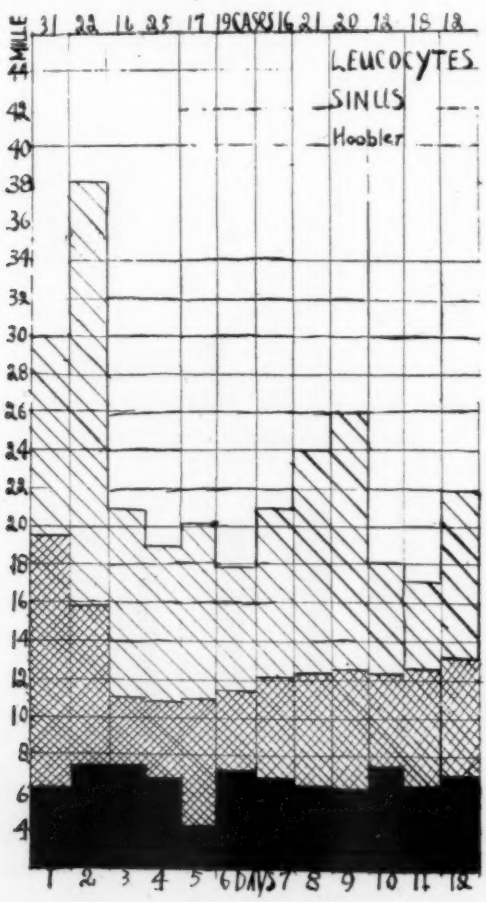

Fig. 5.—White blood count from sinus blood.

3. Hemolysis occurs more easily; the cell membrane is more fragile so that "ghosts" are more frequent, the red blood cells apparently losing their hemoglobin easily though they have more stroma. Fine basophilic granules are more frequent during the first day or two; after that they are rare.

WHITE BLOOD CELLS

We observed the same leukocytosis in the new-born as has been noted by various authors, though this leukoctytosis is by no means constant. The results are shown in Table 5, giving our maximum, average and minimum counts.

TABLE 6.—DIFFERENTIAL SINUS COUNTS

Days	Cases	Neutrophils	Small Lmypho-cytes	Transitionals and Mononuclears	Eosinophils
1	32	70	20	9	1
2	26	62	24	11	1
3	16	58	27	12	1
4	23	54	30	13	1½
5	20	49	34	15	3
6	23	45	37	16	4
7	16	42	42	16	5
8	20	37	45	16	4
9	19	36	45	16	4
10	14	35	46	15	2
11	17	33	47	16	2
12	12	30	48	14	2

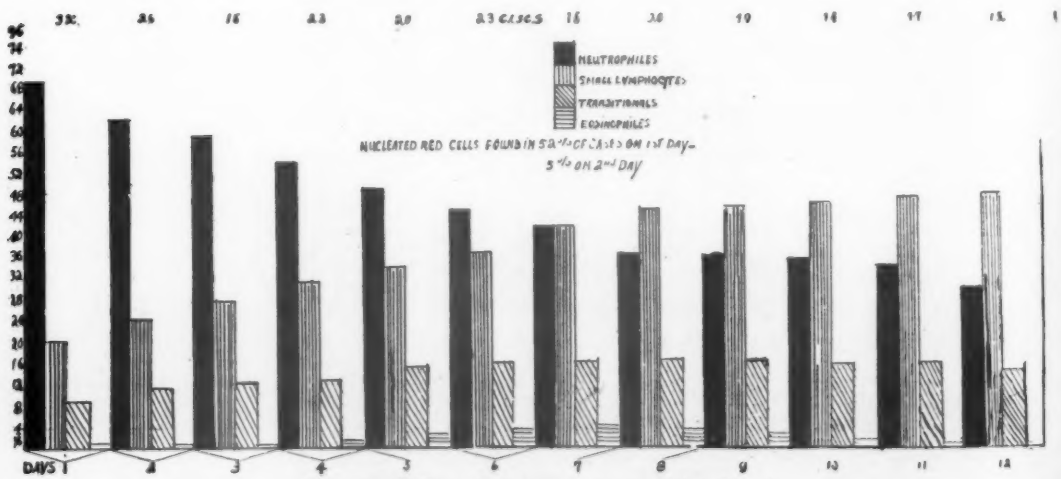

Fig. 6.—Differential count from sinus blood.

These counts correspond with those reported by von Reuss.[10] Although the table of Scibiades is somewhat lower than our findings, the general curve corresponds very closely. Gundobin reports similar findings in early infancy.

10. Von Reuss: The Diseases of the New-Born, p. 41.

The leukocytosis during the first week is definitely due to the polymorphonuclear cells. This continues to be so until the seventh day during which time the polymorphonuclears gradually decline, reaching the same level as the lymphocytes on this day. The lymphocytes during this same period gradually increase and are still increasing at the close of the period of our observation. There is slight increase in the transitional cells which toward the end of our period of observation decrease slightly. The same is true of the eosinophils. These findings are shown in Table 6.

We also compared the total counts between the sinus blood and the blood derived from the periphery. This latter again shows a slight increase over the count from the sinus blood (Table 7).

TABLE 7.—WHITE COUNT OF SINUS AND PERIPHERAL BLOOD COMPARED

Days		Cases	Maximum	Average	Minimum
1.	Sinus	16	27,200	19,400	6,000
	Peripheral	12	21,000	15,500	11,000
2.	Sinus	15	20,000	13,400	7,200
	Peripheral	9	18,000	13,400	10,000
3.	Sinus	6	20,000	13,600	8,100
	Peripheral	8	16,000	11,500	8,500
4.	Sinus	16	15,200	9,600	5,400
	Peripheral	7	17,800	10,670	7,400
5.	Sinus	9	20,000	11,200	5,800
	Peripheral	7	13,400	10,500	6,200
6.	Sinus	16	17,600	11,400	6,000
	Peripheral	6	12,000	10,900	9,800
7.	Sinus	11	21,000	12,100	6,800
	Peripheral	5	13,000	11,600	11,000
8.	Sinus	11	16,000	11,300	6,000
	Peripheral	5	12,400	10,620	9,800

At the present time there is a great deal of discussion as to the classification of the various cells. We are just undertaking a careful investigation of these by using some of the newer vital staining methods and hope to report on this at a later date so that at this time we will not enter into the discussion of the various types of cells found.

Our platelet counts during the first eight days are shown in Table 8.

These figures correspond very nearly to those reported by Dr. Mary E. Morse.[11] Morse found immediately after birth a platelet count which varied from 412,000 to 100,000. We did not find any counts as low as the latter figure, our lowest being 196,000 on the eighth day. Neither were the platelets reduced in cases in which the coagulation was prolonged, nor was there any difference in cases that were markedly jaundiced or those that were slightly or not at all jaundiced. Rebaudi [12] found a very much lower count, 95,000, in the new-born. The

11. Morse: The Blood Platelets in Normal Women, in Obstetrical Patients and in the New-Born, Boston M. & S. J. **166**:448, 1912.

12. Rebaudi: Von Reuss, p. 43.

importance of platelet counts will be referred to in the discussion on coagulation.

CHEMISTRY

As pointed out by Sedgwick there are comparatively few studies on the chemistry of the blood of the new-born. For this reason we have repeated estimations on non-protein nitrogen, urea, uric acid, creatinin,

TABLE 8.—PLATELET COUNTS

Days	Cases	Maximum	Average	Minimum
1	12	396,000	305,000	225,000
2	9	350,000	300,000	270,000
3	8	380,000	306,000	204,000
4	6	436,000	310,000	275,000
5	7	330,000	295,000	220,000
6	6	318,000	278,000	244,000
7	5	318,000	278,000	250,000
8	5	345,000	266,000	196,000

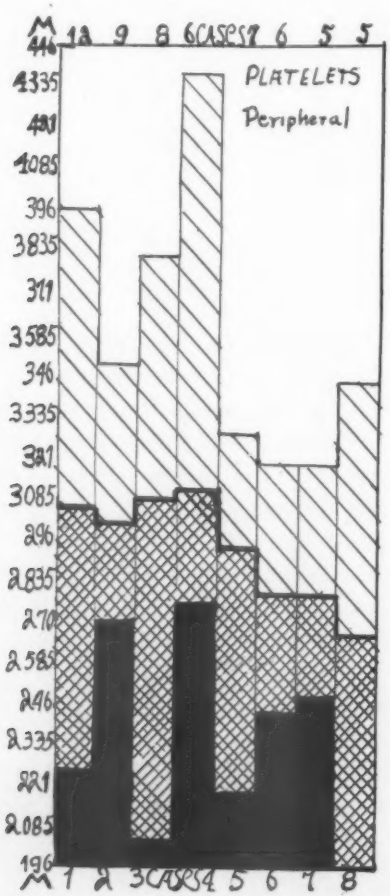

Fig. 7.—Platelet count from peripheral blood.

sugar and carbon dioxid. We have been unable to find any values on the carbon dioxid made on blood of the new-born. Slemons has very clearly demonstrated that the placenta acts as a semipermeable membrane for most of the constituents of the blood so that the estimations of the maternal and fetal blood are approximately the same at the time of birth.

Table 9 gives our findings for the first twelve days.

TABLE 9.—CHEMISTRY OF BLOOD OF NEW-BORN

Day		Nonprotein Nitrogen, Mg. per 100 C.c.	Urea Nitrogen, Mg. per 100 C.c.	Uric Acid, Mg. per 100 C.c.	Creatinin, Mg. per 100 C.c.	Sugar, per Cent. per 100 C.c.	Carbon Dioxid, Vol. % per 100 C.c.
0.	Minimum	32	16	2.07	1.35	0.043	38.5
	Average	35	18	3.18	1.41	0.052	52.5
	Maximum	40	20	4.54	1.49	0.062	63.3
1.	Minimum	34	17	2.12	1.48	0.054	38.5
	Average	40.6	20.4	3.27	1.72	0.066	55.7
	Maximum	52	26	4.87	2.00	0.081	64.3
2.	Minimum	28	14	1.40	1.34	0.045	53.8
	Average	32.7	16.3	2.46	1.54	0.051	63.4
	Maximum	36	18	3.42	1.69	0.060	69.2
3.	Minimum	30	15	2.06	1.23	0.055	51.9
	Average	32.7	16.3	3.06	1.42	0.068	55.7
	Maximum	36	18	5.00	1.64	0.075	62.4
4.	Minimum	26	13	1.47	1.24	0.052	45.3
	Average	35.5	17.5	2.59	1.49	0.082	59.7
	Maximum	53.1	24	4.00	2.07	0.133	67.2
5.	Minimum	28	15	1.65	1.24	0.039	55.7
	Average	32.5	16.5	2.95	1.46	0.064	59.5
	Maximum	38	20	4.67	1.59	0.080	63.3
6.	Minimum	27	10	1.73	1.14	0.056	41.1
	Average	32.4	15.8	2.33	1.37	0.078	58.1
	Maximum	37	19	3.30	1.75	0.101	66.2
7.	Minimum	25	13	1.50	1.25	0.058	55.7
	Average	31.8	15.8	2.02	1.44	0.077	62.0
	Maximum	39	18	2.52	1.68	0.123	74.8
8.	Minimum	23	12	1.57	0.99	0.062	46.2
	Average	31.8	16.4	2.08	1.21	0.085	57.4
	Maximum	38	20	2.50	1.42	0.024	62.4
9.	Minimum	31	15	1.95	1.30	0.071	57.6
	Average	31.7	15.5	1.97	1.34	0.078	60.1
	Maximum	32.4	16	2.00	1.39	0.086	61.4
10.	Minimum	23	12	1.22	1.06	0.066	56.7
	Average	28.7	14	2.15	1.16	0.077	59.5
	Maximum	37	15	3.12	1.35	0.085	64.3
11.	Minimum	26	13	1.42	1.11	0.092	55.7
	Average	30.8	15.2	1.84	1.28	0.099	60.5
	Maximum	35	17	2.38	1.50	0.112	66.2
12.	Minimum	24.1	12.7	1.45	1.00	0.072	50.7
	Average	27.6	13.6	1.72	1.11	0.083	58.3
	Maximum	31	15	2.06	1.29	0.097	63.6

Nonprotein nitrogen, urea, sugar and uric acid by Folin method; carbon dioxid, Van Slyke; creatinin by Folin and Wu method.

In comparing our results with the observations made by Schlutz and Pettibone [13] we find our figures to be somewhat higher than theirs. The figures of Sedgwick et al (ibid.) are higher than ours except the

13. Schlutz and Pettibone: Am. J. Dis. Child. **10**:206 (Sept.) 1916.

urea nitrogen which falls below our findings. Our blood sugar findings are somewhat lower than those of Sedgwick et al., although comparative curves would be almost parallel. Our only explanation of the low sugar findings in this series is that the infants were breast fed and received no supplementary food except water. They were on four hour feedings and the estimations were taken just before nursing.

The graphic curves which are appended show a very definite drop in the nonprotein nitrogen, urea and creatinin during the first few days and show a definite rise in the sugar and carbon dioxid curve. There are comparatively few estimations on creatinin during the first few weeks. Our curves show very definitely that during this period there is a high body catabolism as the creatinin is only of endogenous origin.

CALCIUM

Howland and Marriott [14] have shown that the calcium values in blood for infants are essentially those found in the adult. They examined placental blood and blood from six normal infants. They did not state the age of these infants but we presume that none of them were new-born. In their comparison they found very little variation of the calcium content between the placental blood (from 10.7 to 11 mg. per hundred c.c. blood), normal adult blood (from 9.2 to 11.2 mg. per hundred c.c blood), and normal infant blood (from 10.8 to 11.5 mg. per hundred c.c. blood).

In a recent study by Jones and Nye [15] from our laboratories, the distribution of calcium and phosphoric acid in the blood of normal children was found to average in whole blood 9.4 mg. per hundred c.c.; corpuscles, 8.7; plasma, 10. So far as we are aware, no data have been published on the distribution of the calcium in the blood of the new-born.

Table 10 gives the results of sixty-eight determinations on twenty-two infants ranging in age from 1 to 12 days.

The technic followed for these determinations was the same as that described by Jones and Nye, except that 3 c.c. corpuscles were laked with an equal volume of distilled water and 5 c.c. of this was added to 20 c.c. trichloracetic acid instead of 5 c.c. of a one to three dilution. This was found to be necessary on account of the low calcium content of the corpuscles of the new-born. The blood was collected about four hours after the last feeding, from 15 to 20 c.c. being taken from the longitudinal sinus. From one to five determinations were made on each of twenty-two infants, twelve boys and ten girls. The first sample of blood was taken as soon after birth as possible, usually within twelve hours, and at intervals of three or four days thereafter as long as the infant was in the hospital.

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14. Howland and Marriott: Tr. Am. Ped. Soc. **28**:202, 1916.
15. Jones and Nye: J. Biol. Chem. **47**:321 (July) 1921.

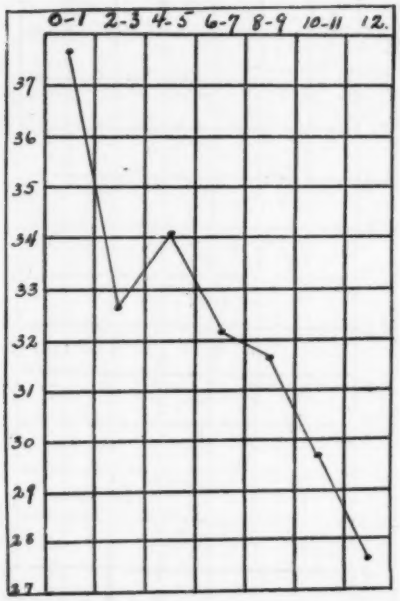

Fig. 8.—Nonprotein nitrogen, mg. per hundred c.c.

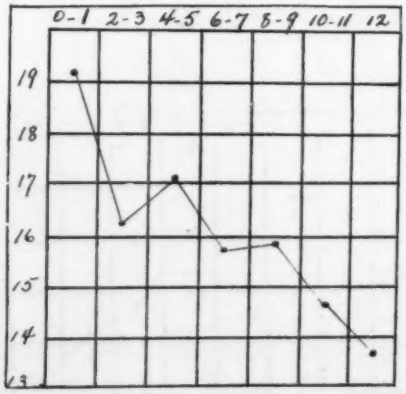

Fig. 9.—Urea nitrogen, mg. per hundred c.c.

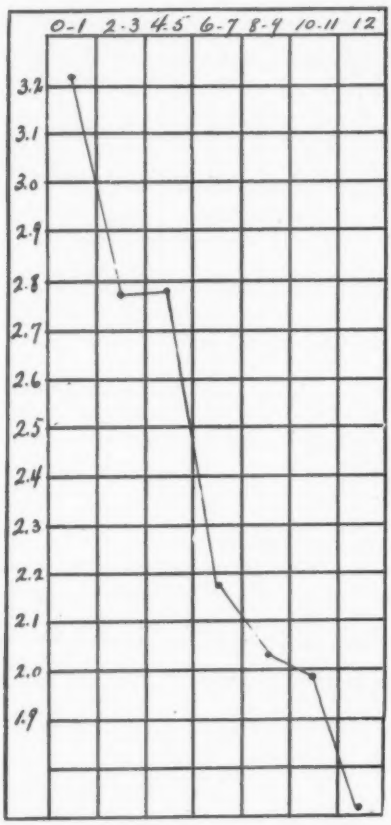

Fig. 10.—Uric acid, mg. per hundred c.c.

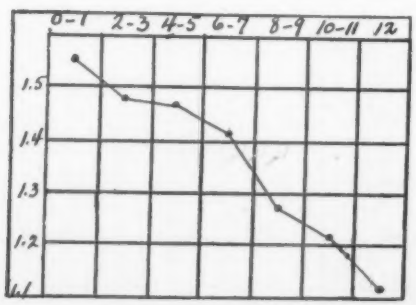

Fig. 11.—Creatinin, mg. per hundred c.c.

An examination of Table 10 shows that the average calcium content of the plasma is higher in the new-born than in older children. This is in agreement with the findings of Meiggs, Blatherwick and Cary [16] who showed that in heifers the calcium content of the blood plasma

TABLE 10.—A. CALCIUM CONTENT OF THE BLOOD OF NORMAL INFANTS FROM BIRTH TO 12 DAYS OF AGE *

Sex	Age, Days	No. of Determination Included in	Average Hematocrit Reading, per Cent.	Average Calcium Values per 100 C.c.		
				Whole Blood, Mg.	Corpuscles, Mg.	Plasma, Mg.
Boys	0-2	5	55.1	8.7	5.8	12.1
	2-4	6	50.5	8.9	5.4	12.3
	4-6	6	49.1	8.8	5.4	12.3
	6-8	6	45.7	9.0	5.2	12.3
	8-10	6	47.2	8.9	5.1	12.3
	10-12	6	40.8	9.4	5.0	12.3
Average	0-12	35	48.6	9.0	5.3	12.3
Girls	0-2	6	54.8	8.2	4.9	12.2
	2-4	6	49.2	8.5	4.9	12.0
	4-6	5	49.0	8.6	4.7	12.2
	6-8	5	45.7	8.8	4.7	12.3
	8-10	6	43.9	8.9	4.5	12.1
	10-12	5	42.8	9.1	4.7	12.5
Average	0-12	33	47.4	8.7	4.7	12.2
Boys and girls	0-2	11	55.0	8.4	5.4	12.2
	2-4	12	49.9	8.7	5.2	12.2
	4-6	11	49.1	8.7	5.1	12.3
	6-8	11	45.7	8.9	5.0	12.3
	8-10	12	45.5	8.9	4.8	12.3
	10-12	11	41.9	9.3	4.9	12.4
Average	0-12	68	48.0	8.8	5.0	12.3

B. AVERAGES AND VARIATIONS IN THE CALCIUM CONTENT OF THE BLOOD OF NORMAL INFANTS RANGING IN AGE FROM 4 HOURS TO 12 DAYS, AND OF NORMAL CHILDREN FROM 4 WEEKS TO 14 YEARS *

	Calcium per 100 C.c.			Red Blood Cells, per Cent.
	Whole Blood, Mg.	Corpuscles, Mg.	Plasma, Mg.	
Low				
Infants	8.1	4.2	11.4	37.1
Children	5.6	5.2	5.5	30.1
Average				
Infants	8.8	5.0	12.3	48.0
Children	9.4	8.7	10.0	38.2
High				
Infants	10.3	6.9	13.2	64.5
Children	12.4	12.0	12.4	44.0

* Analyses made by Martha R. Jones, Ph.D.

is highest at birth and tends to become lower with advance in age up to six months. It is interesting to note the constancy of the plasma volumes throughout the series, each determination varying less than 0.4 mg. per hundred c.c. from the general average (12.3 mg. per

16. Meiggs, Blatherwick and Cary: J. Biol. Chem. **37**:1, 1919.

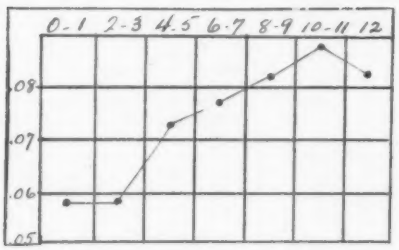

Fig. 12.—Sugar per cent. per hundred c.c.

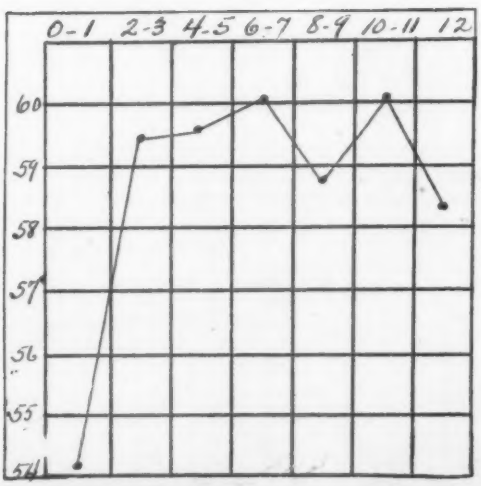

Fig. 13.—Carbon dioxid volume per cent. per hundred c.c.

100 c.c.). Apparently in man the drop in the plasma content does not occur during the first twelve days of life. The value for whole blood (8.8 mg. per hundred c.c.) is slightly less than that (9.5 mg.) reported by Brown, MacLachlan and Simpson [17] in normal infants under 1 year of age while the average content of corpuscles (5 mg.) is markedly less than that (8.7 mg.) found by Jones and Nye in older children.

In general, the corpuscle values tend to decrease slightly during the first few days. The difference, however, in the majority of cases was well within the limits of experimental error and too much emphasis should not be laid on it. On the other hand, there is a tendency for the whole blood values to increase. This is what one would expect with relatively constant values for plasma and corpuscles and a marked decrease in the percentage of cells. The calcium values in the new-born tend to run higher in boys than girls, as was pointed out by Jones and Nye in the case of older children.

These calcium estimations go to show very clearly that in the blood of the new-born, calcium is rarely at fault in any problem related to hemorrhage of the new-born, except in those cases of obstructive jaundice in which case the calcium is bound with the bile salts and is, therefore, unavailable for entering into the process of coagulation.

COAGULATION

Many methods for estimating coagulation time have been developed, but all these methods so far have employed blood derived from superficial puncture. Rodda [18] has reviewed various methods for estimating coagulation time. He has proposed a very useful clinical method for the estimation of blood obtained from punctures in which "all factors, such as depth and site of puncture, size and sequence of the drops, temperature, air currents and the effect of foreign bodies in contact with the blood were duly considered." However, any method which uses a puncture wound must of necessity have certain inherent errors. As has been pointed out by Lee and White [19] and Lee and Vincent,[20] the only accurate methods for determining coagulation time are those in which the blood is obtained by venipuncture. The advantages of obtaining the blood directly from a vein or from the longitudinal sinus, as we carried it out, are that there is no contact with tissue or skin. It has been shown by these authors that blood drawn from superficial punctures clots in varying time depending on the depth of the puncture wound.

17. Brown, MacLachlan and Simpson: Am. J. Dis. Child. **19**:413, 1920.
18. Rodda: Am. J. Dis. Child. **19**:269 (April) 1920.
19. Lee and White: Am. J. M. Sc. **145**:495 (April) 1913.
20. Lee and Vincent: Arch. Int. Med. **13**:398 (April) 1914.

TABLE 11.—Cases with Prolonged Coagulation Time and Diminished Prothrombin without Hemorrhage

Name	Age, Days	Coagulation Time, Min.	Prothrombin		Age, Days	Coagulation Time, Min.	Prothrombin		Age, Days	Coagulation Time, Min.	Prothrombin		Age, Days	Coagulation Time, Min.	Prothrombin		Age, Days	Coagulation Time, Min.	Prothrombin	
			Tube	Min.			Tube	Min.			Tube	Min.			Tube	Min.			Tube	Min.
Baby AA	1	17	2	0	3	25	2	0												
			3	11			3	0												
			4	12			4	60 (sl)												
			5	18			5	27												
Baby BB	2	43	2	25	4	38	2	44	6	17	2	17	9	15	2	19	13	9	2	9
			3	30			3	38			3	16			3	16			3	10
			4	30			4	40			4	15			4	15			4	10
			5	31			5	42			5	25			5	16			5	15
Baby A	1	18	2	0	4	32	2	34	6	14	2	13								
			3	19			3	28			3	10								
			4	17			4	38			4	15								
			5	15			5	38			5	20								
Baby CC	3	20	0	0	6	24	2	10	8	13	2	9								
							3	11			3	10								
							4	12			4	11								
							5	17			5	11								
Baby J	1	23	2	28	4	16	2	14	6	8	2	0	8	12	2	0	11	17	2	10
			3	28			3	14			3	11			3	7			3	12
			4	18			4	11			4	14			4	9			4	13
			5	22			5	23			5	16			5	11			5	15
Baby H	1	16	2	20	4	24	2	0	6	7	2	10	8	8	2	14	11	12	2	6
			3	20			3	30			3	7			3	10			3	6
			4	21			4	15			4	8			4	12			4	11
			5	30			5	15			5	9			5	14			5	12
Baby DD	1	29	0	0	3	17	2	0	6	7	2	6	8	12	0	0				
							3	36			3	5								
							4	26			4	6								
							5	28			5	7								
Baby EE	1	28	2	14	3	21	0	0	8	23										
			3	10																
			4	10																
			5	15																

TABLE 12.—Comparative Table of Coagulation and Prothrombin with Amount of Bilirubin in Plasma

Name	Age, Days	Coagulation Time, Min.	Prothrombin		Bilirubin	Age, Days	Coagulation Time, Min.	Prothrombin		Bilirubin	Age, Days	Coagulation Time, Min.	Prothrombin		Bilirubin	Age, Days	Coagulation Time, Min.	Prothrombin		Bilirubin	Age, Days	Coagulation Time, Min.	Prothrombin		Bilirubin	Age, Days	Coagulation Time, Min.	Prothrombin		Bilirubin	Age, Days	Bilirubin
			Tube	Minutes				Tube	Minutes				Tube	Minutes				Tube	Minutes				Tube	Minutes				Tube	Minutes			
Baby L	½	10	2	12	15.7	2	10	2	17	40.0	4	12	2	15	41.1	7	13	2	7	50.5	9	9	2	11	44.1	11	..	2	..	34.8	14	34.8
			3	10				3	13				3	9				3	9				3	12				3				
			4	11				4	17				4	11				4	14				4								
			5	15				5	20				5	14				5	12				5	15				5				
Baby V	1	13	...	..	13.9	3	10	2	22	29.6	5	6	2	8	17.4	8	19	2	11	15.7	10	11	2	13	14.2	12	..	..	..	12.0		
			3	13				3	27				3	9				3	9				3	14								
			4	18				4	29				4	10				4	12				4	20								
			5	23				5	29				5	14				5	13				5	20								
Baby W	1	9	2	0	12.2	3	14	2	19	19.1	5	5	2	16	26.1	8	..	2	9	12.2	10	11	2	12	12.2	12	..	..	..	5.2		
			3	15				3	29				3	14				3	10				3	13								
			4	17				4	29				4	14				4	10				4	18								
			5	17				5	0				5	24				5	11				5	18								
Baby M	2	10	2	13	12.2	4	10	2	17	24.4	6	13	2	13	22.4	9	15	2	11	17.4	11	11	2	14	13.9							
			3	11				3	26				3	9				3	10				3	17								
			4	11				4	34				4	11				4	10				4	19								
			5	12				5	0				5	11				5	18				5	25								
Baby X	2	12	2	15	10.4	4	12	2	15	25.3	7	14	2	12	20.9	9	15	2	10	15.7	11	8	2	19	12.2							
			3	12				3	12				3	11				3	11				3	15								
			4	14				4	14				4	10				4	12				4	17								
			5	22				5	22				5	16				5	12				5	24								
Baby T	2	..	...	..	8.7	4	12	2	17	12.2	6	11	2	8	13.9	9	..	..	..	7.0	11	..	..	..	5.7							
			3	13				3	18				3	9																		
			4	17				4	17				4	10																		
			5	20				5	20				5	16																		
Baby U	2	..	...	..	10.4	4	10	2	15	13.9	6	5	2	7	19.1	9	12	2	7	5.2	11	12	2	10	5.2	13	14	2	17			
			3	17				3	17				3	9				3	9				3	12				3	21			
			4	20				4	20				4	9				4	9				4	17				4	22			
			5	21				5	21				5	11				5	9				5	17				5	24			

TABLE 13.—Comparative Table of Coagulation and Prothrombin

Name	Age, Days	Coagulation Time, Min.	Retraction Time	Prothrombin		Age, Days	Coagulation Time, Min.	Retraction Time	Prothrombin		Age, Days	Coagulation Time, Min.	Retraction Time	Prothrombin		Age, Days	Coagulation Time, Min.	Retraction Time	Prothrombin		Age, Days	Coagulation Time, Min.	Retraction Time	Prothrombin		Age, Days	Coagulation Time, Min.	Retraction Time	Prothrombin	
				Tube	Minutes				Tube	Minutes				Tube	Minutes				Tube	Minutes				Tube	Minutes				Tube	Minutes
Baby A	2	18	G	2	0	4	32	G	2	34	6	14	G	2	13														
				3	19				3	28				3	10														
				4	17				4	38				4	15														
				5	15				5	38				5	20														
Baby B	1	13	G	2	15	7	6	G	2	0	9	13	G	2	10	12	9	G	2	9									
				3	8				3	12				3	11				3	10									
				4	9				4	10				4	11				4	11									
				5	13				5	9				5	12				5	11									
Baby C	3	11	G	2	12	9	11	G	2	15	12	7	G	2	6														
				3	13				3	12				3	8														
				4	13				4	13				4	8														
				5	15				5	18				5	8														
Baby D	1	14	G	2	0	8	11	G	2	12	10	9	G	2	8														
				3	32				3	9				3	8														
				4	30				4	11				4	8														
				5	32				5	14				5	10														
Baby E	5	18	G	2	0	7	11	G	2	0	9	11	G	2	13														
				3	18				3	11				3	11														
				4	20				4	12				4	11														
				5	22				5	12				5	15														
Baby F	1	16	G	2	28	4	19	G	2	11	6	12	G	2	0	8	12	G	2	10									
				3	24				3	12				3	5				3	10									
				4	24				4	13				4	7				4	11									
				5	25				5	15				5	8				5	12									
Baby G	1	17	G	2	15	3	16	G	2	25	5	11	G	2	35	8	10	G	2	0									
				3	14				3	14				3	15				3	9									
				4	12				4	15				4	17				4	11									
				5	15				5	17				5	24				5	15									
Baby H	6	7	G	2	10	8	9	G	2	14	11	12	G	2	6														
				3	7				3	10				3	6														
				4	8				4	12				4	11														
				5	9				5	14				5	12														
Baby I	5	11	G	2	24	7	10	G	2	0	10	9	G	2	7														
				3	22				3	8				3	4														
				4	20				4	10				4	6														
				5	22				5	15				5	9														
Baby J	1	23	G	2	28	4	17	G	2	14	6	9	G	2	0	8	12	G	2	0	11	17	G	2	10				
				3	28				3	14				3	11				3	7				3	12				
				4	18				4	11				4	14				4	9				4	13				
				5	22				5	23				5	16				5	11				5	14				
Baby K	1	11	G	2	12	3	15	G	2	15	5	7	G	2	0	8	10	G	2	11	12	8	G	2	10				
				3	11				3	14				3	7				3	6				3	12				
				4	9				4	15				4	9				4	10				4	14				
				5	10				5	20				5	11				5	14				5	15				

Baby L	½	10	G	2	12	2	10	G	2	17	4	12	G	2	15	7	13	G	2	7	9	9	G	2	11	11	7	G	2	12
				3	10				3	13				3	9				3	9				3	12				3	14
				4	11				4	17				4	11				4	11				4	14				4	16
				5	15				5	20				5	14				5	12				5	15				5	20
Baby M	2	10	G	2	13	4	10	G	2	17	6	13	G	2	13	9	15	G	2	11	11	11	G	2	14	13	14	G	2	15
				3	11				3	26				3	9				3	10				3	17				3	18
				4	11				4	34				4	11				4	10				4	19				4	19
				5	12				5	0				5	11				5	18				5	25				5	0
Baby N	2	15	G	2	0	7	15	G	2	18	12	11	G	2	9															
				3	25				3	17				3	8															
				4	19				4	20				4	10															
				5	17				5	20				5	12															
Baby O	13 Hrs.	16	G	2	13	3	14	G	2	25	5	18	G	2	0	8	15	G	2	0										
				3	14				3	17				3	28				3	14										
				4	17				4	22				4	27				4	15										
				5	18				5	0				5	28				5	16										
Baby P	4	18	G	2	15	9	12	G	2	8	11	0	G	2	8															
				3	14				3	8				3	8															
				4	0				4	9				4	9															
				5	0				5	0				5	0															
Baby Q	2	15	G	2	0	4	11	G	2	17	6	11	G	2	14	8	7	G	2	11	10	9	G	2	10	13	11	G	2	9
				3	21				3	17				3	11				3	11				3	10				3	10
				4	23				4	18				4	15				4	12				4	11				4	10
				5	0				5	0				5	30				5	14				5	15				5	18
Baby R	2	20	G	2	0	4	8	G	2	17	6	12	G	2	20	8	12	G	2	27										
				3	20				3	12				3	19				3	21										
				4	21				4	20				4	28				4	20										
				5	30				5	24				5	29				5	25										
Baby S	2	16	G	2	0	5	13	G	2	23	7	13	G	2	11	9	9	G	2	8	12	15	G	2	8					
				3	15				3	14				3	12				3	9				3	9					
				4	18				4	16				4	13				4	9				4	10					
				5	25				5	22				5	15				5	12				5	11					
Baby T	4	12	G	2	17	6	11	G	2	8	11	12	G	2	8															
				3	13				3	9				3	9															
				4	17				4	10				4	10															
				5	20				5	10				5	12															
Baby U	4	10	G	2	15	6	5	G	2	7	9	12	G	2	7	11	12	G	2	10	13	14	G	2	17					
				3	17				3	9				3	9				3	12				3	21					
				4	20				4	9				4	9				4	17				4	22					
				5	21				5	11				5	9				5	17				5	24					
Baby V	1	13	G	2	0	3	10	G	2	22	5	6	G	2	8	8	19	G	2	11	10	11	G	2	13					
				3	13				3	27				3	9				3	9				3	14					
				4	18				4	29				4	10				4	12				4	20					
				5	23				5	29				5	14				5	13				5	20					
Baby W	1	9	G	2	0	3	14	G	2	19	5	5	G	2	16	7	..	G	2	9	10	11	G	2	12					
				3	15				3	29				3	14				3	10				3	13					
				4	17				4	29				4	14				4	10				4	18					
				5	17				5	0				5	24				5	11				5	18					
Baby X	2	12	G	2	26	4	12	G	2	15	7	14	G	2	12	9	15	G	2	10	11	8	G	2	19					
				3	29				3	12				3	11				3	11				3	15					
				4	0				4	14				4	10				4	12				4	17					
				5	0				5	22				5	16				5	12				5	24					

The method used by us was to obtain blood from the longitudinal sinus with an all glass luer syringe which had been sterilized carefully, washed out with physiologic solution of sodium chlorid and then coated with sterile oil (liquid petrolatum). The blood was immediately delivered in 2 c.c. quantities into test tubes measuring 21 mm. in diameter which had been previously cleansed in bichromate and dried with alcohol and ether. The tubes were allowed to stand for five minutes before the first test was made, as any coagulation which occurred in less time than this was found to be due to lack of technic, difficulty in getting the blood from the sinus, introduction of air bubbles, etc. After a five minute period the tubes were rotated every thirty seconds and the coagulation time was determined at the point when the tubes could be inverted without the clot slipping. This was taken as the end point.

Our results are shown in Figures 14 and 15.

This shows very clearly that during the first four days there is a definite prolongation in the coagulation time. This is a very important observation, as we feel that it shows clearly that during these first few days there is a definite and fairly constant condition in the blood of the new-born which favors the so-called hemorrhagic condition of the new-born. As has been shown by various studies in isolated cases, the hemorrhagic condition of the new-born may be divided into several classes. Whipple has shown in certain cases of hemorrhage of the new-born that the prothrombin element is a causative factor. We have made the same observation in the study of a case of hemorrhagic disease of the new-born by Gelston.[21] Whipple has also shown in certain cases of liver disturbance that the fibrogen factor may be at fault, and in cases of obstructive jaundice of the new-born the calcium factor is undoubtedly partly at fault. In cases of sepsis, the antithrombin factor has been shown by Whipple to be involved.

However, we wish to emphasize the fact that the blood of the new-born normally has a prolonged coagulation time, whether hemorrhage appears or not. The appearance of hemorrhage, we believe, depends on whether this factor is exaggerated or not. We have found the coagulation time prolonged as long as twenty, thirty and forty minutes without the appearance of any hemorrhage or increase in bleeding time. This average (bleeding time) is from one and one-half to three minutes in all cases.

As has already been pointed out, the calcium content of the blood of the new-born is greater than that of older infants. We have also pointed out that the platelets are not diminished. During this same period of prolonged coagulation time the serum bilirubin curve is also increased, but it does not seem to affect the coagulation time in cases of marked jaundice. Table 12 bears out this statement. These are

21. Gelston: Am. J. Dis. Child. **22**: 351 (Oct.) 1921.

representative cases with marked increase in coagulation or decrease in prothrombin element as compared with our normal curves.

We have investigated the prothrombin factor and the following curves, we believe, prove that during the period in which the coagulation time is prolonged the prothrombin element is definitely diminished. The curves in all the tubes to which calcium was added, varying in amount from 2 to 5 drops according to Howell's method of recalcifying oxalated blood by the addition of the optimum amount of calcium

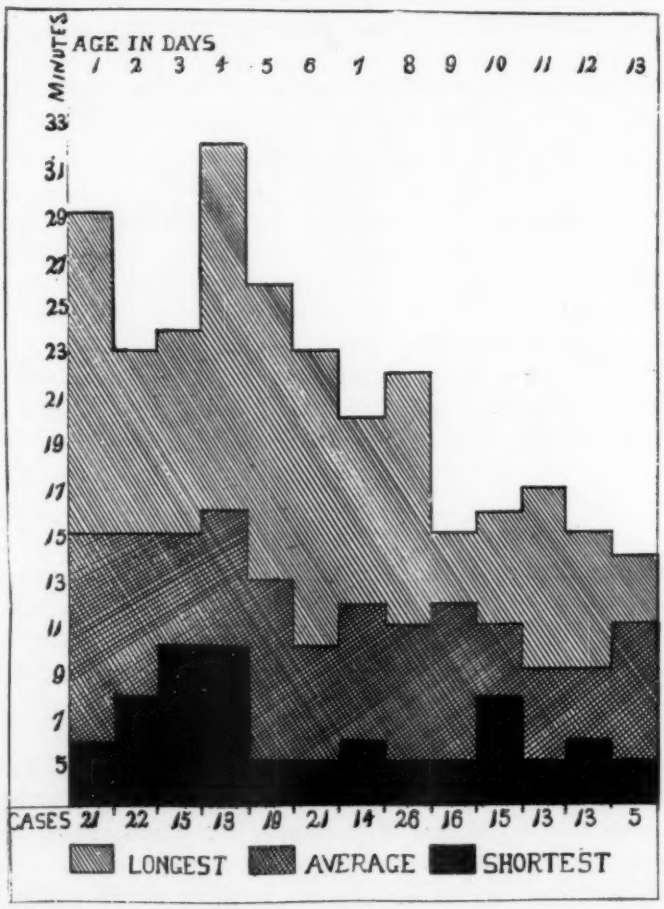

Fig. 14.—Coagulation.

chlorid, show a diminution of the prothrombin element during the first four or five days. After this time the prothrombin factor apparently reaches normal levels though it may be delayed in certain cases for several days longer. We have attempted to show that this is due to a qualitative defect in the platelets as was shown by Minot and Lee [22] to exist in the case of hemophilia.

22. Minot and Lee: Arch. Int. Med. **18**:474 (Oct.) 1916.

Table 14, we believe, shows that the same qualitative defect exists in the platelets of the new-born during these first few days.

The blood of the new-born did not show any defect in retractility of the clot and there was no fibrinolysis. This is exactly the condition which exists in hemophilia. After the blood clots in hemophilia the retraction of the clot is normal and there is no fibrinolysis. We feel confident, therefore, that the factor involved in the ordinary case of hemorrhage of the new-born is due to diminution in the prothrombin element which is mainly derived from the platelets and the defect is essentially a qualitative one of the platelets. The platelets apparently

TABLE 14.—Difference in Prothrombin and Platelets on Various Days

Name	Age	Clear		Very Clear		4 Drops		6 Drops		8 Drops	
Baby FF	2	2	17	2	0						
		3	10	3	0						
		4	9	4	30	4	10	4	20	4	17
		5	9	5	18	5	20	5	24	5	0
Baby GG	4	2	0	2	0						
		3	0	3	0						
		4	34	4	50	4	25	4	21	4	15
		5	20	5	45	5	23	5	20	5	15
Baby HH	4	2	..	2	0						
		3	..	3	0						
		4	..	4	0	4	27	4	0	4	0
		5	..	5	1 hr.	5	23	5	23	5	20
Baby II	7	2	17	2							
		3	10	3	22						
		4	9	4	18	4	9	4	8	4	7
		5	9	5	22	5	6	5	8	5	8
Baby JJ	9	2	14	2	16						
		3	11	3	16						
		4	9	4	12	4	6	4	8	4	6
		5	9	5	15	5	6	5	6	5	6
Baby KK	10	2	10	2	18						
		3	10	3	17						
		4	10	4	15	4	5	4	5	4	5
		5	8	5	17	5	5	5	5	5	6
Baby LL	10	...	..	2	0						
				3	16	3	8	3	8	3	7
				4	18	4	8	4	8	4	8
				5	20						

obtain their normal prothrombin element very rapidly after birth. It is during the first three or four days that most cases of hemorrhage of the new-born occur and it is during the same period that the platelets are defective in their prothrombin element.

We have attempted to demonstrate urobilin in the stools of the new-born. Our technic was as follows: Soft paper diapers were used in the collection of stools and the entire mass from which all of the uncontaminated paper had been removed was dropped immediately after being passed into a specimen jar containing about 150 c.c. of a dilute hydrochloric acid solution (1 c.c. concentrated hydrochloric acid solution to 100 c.c. distilled water). The stools were collected in two day periods, the dilute acid being used to prevent bacterial decomposi-

tion. The entire mass (paper and stool) was macerated in the original jar with a spatula and pestle and the liquid portion strained through a Büchner funnel. The residue was then ground in a mortar until of even consistency and added to the liquid that had been strained off. After thorough mixing, the liquid portion was again strained through the funnel into a graduate, as much as possible being expressed from the paper pulp. The volume of pulp was then determined, and one-half of the amount calculated as stool mixture was added to that measured in the graduate. In order to approximate the amount of water necessary to make a pulp of like consistency, a measured amount of water was added to some of the paper diapers and treated as in the stool mixture. The excess of water was expressed and the volume required to make a known amount of pulp calculated. It was found that approximately 50 c.c. of water were used in making 100 c.c. of pulp.

The method for the determination of urobilin and urobilinogen was that described by Wilbur and Addis.[23] So far we have been unable to show the presence of any urobilin in the stools of new-born infants during the first twelve days of life, nor have we so far been able to demonstrate urobilin in stools of infants up to ten weeks of age, although in older children it is easy to demonstrate its presence. This still leaves open the question of what becomes of the red blood cells which diminished so rapidly during these first few days of life. We hope by further studies to be able to answer some of the questions which so far have not been satisfactorily solved by these and other studies on the new-born.

BILE PIGMENT IN THE PLASMA

The problem of icterus neonatorum is one which has received a great deal of attention, but so far there are many conflicting theories regarding its cause. At this time we do not intend to go into the discussion of the various phases but simply to report the method of testing for bile pigment in blood plasma which we believe is more accurate from a qualitative as well as quantitative standpoint than any other method thus far advanced for routine study.

Method.—The sample of freshly drawn blood is mixed at once with a sufficient amount of oxalate and centrifuged at high speed for thirty minutes. This removes the platelets, and since the blood was drawn before feeding there is little clouding from fat. The resulting supernatant plasma is usually clear, with varying shades of red to brown from the various chromatic elements present. Three c.c. of the plasma or serum are withdrawn in a calibrated pipet and transferred drop by drop into a 25 c.c. volumetric flask containing a little less than 22 c.c. of the acid alcohol standard solution (this solution is made up to 30 c.c. of concentrated hydrochloric acid with sufficient 95 per cent. alcohol to make 2,000 c.c.). After delivering the sample of plasma, the volumetric flask is filled to the 25 c.c. level with the standard solution. On standing (from twelve to twenty-four hours), an emerald green color

23. Wilbur and Addis: Arch. Int. Med. **13**:235 (March) 1914.

develops which reaches its maximum intensity by twenty-four hours. From this place in the procedure the method follows that of Hooper and Whipple.[24] The solution is filtrated and read in the Hellige's colorimeter against a standard wedge made up of gelatin, copper sulphate and India ink. The standard wedge is the same as is used in connection with the pigment analysis of the bile in the routine bile fistula work at the Hooper Foundation. This wedge has been standardized against pure bilirubin (Kaulbaum), bilirubin from gallstones and crystalline bilirubin from dog's bile. A table for the values in milligrams of bilirubin corresponding to the colorimeter readings allows a quick determination of pigment in the samples of plasma.

In arriving at the above routine method, several difficulties were apparent. It was certain that the blood would not contain the same amount of pigment as the bile and it was possible that the precipitation of the protein might occlude considerable pigment. Accordingly, the proportion of plasma to acid alcohol could not be the same as the proportion of bile to acid alcohol. It was found after repeated trials that 3 c.c. of plasma in 25 c.c. of acid alcohol developed sufficient color to be read against the bile pigment standard wedge and yet did not require the withdrawal of too large amounts of blood. This proportion insures thorough precipitation of the proteins and sufficient filtrate to make several estimations. In fact, where only 1 c.c. of plasma is obtainable, the proportion of 1 c.c. in 10 c.c. of acid alcohol will give approximate results. For accuracy, the 3 c.c. sample is preferable.

The development of the color can, of course, be hastened by heating or by adding nitric acid. Furthermore, just as with the bile, the pigment, after forty-eight hours, approaches the blue field which is indicative of oxidation past biliverdin to the bilicyanin. As a routine, the color was developed at room temperature while exposed to the light of the laboratory.

Although this color reaction is specific for bile pigments it was not certain that when applied to the blood the method was sufficiently accurate for quantitative analysis. However, it was found that if plasma from normal controls (serum from normal adults) which gave only the white protein precipitate with no color development, was used to dilute icteric plasmas the diluted specimens could be estimated with the proportional amount of pigment. Furthermore, when known amounts of bile containing estimated quantities of bile pigment were added to either icteric plasmas or non-icteric plasmas the resulting mixtures when estimated gave the theoretical calculation well within the delicacy of the method. The bile used was both human bile from operative cholecystostomy cases and dog bile from the bile fistula animals in the Hooper Laboratory.

Table 15 shows the accuracy of the method in the recovery of known amounts of pigment.

Although there are numerous qualitative tests for bilirubin, few of these are obtainable for quantitative methods. In most cases, the tests

apply to the bile or urine. A method for quantitative estimation of bile pigment in the blood, while much to be desired, has not as yet to our knowledge been perfected for routine study.

Further, the quantitative estimation of substances by the colorimetric method always presents a great many difficulties and at best involves considerable experimental error. This would seem particularly true concerning the estimation of bilirubin in the blood where, as is known, there are present not only the related hemoglobin derivatives but also the lipochromes.

TABLE 15.—BILE PIGMENT IN PLASMA

Sample	Proportion	Calculated Reading	Theoretical Reading
1. Normal adult plasma (control).....	3.0 c.c. in 25 c.c. St.	No color	No color
2. Dog bile	0.3 c.c. in 21 c.c. St.	30	30
3. Icteric plasma	3.0 c.c. in 25 c.c. St.	20	20
4. Normal adult plasma.....	2.9 c.c. in 25 c.c. St.		
Dog bile	0.1 c.c. in 25 c.c. St.	10	10
5. Icteric plasma	2.9 c.c. in 25 c.c. St.		
Dog bile	0.1 c.c. in 25 c.c. St.	31	30
6. Normal adult plasma.....	1.0 c.c. in 25 c.c. St.		
Icteric plasma	2.0 c.c. in 25 c.c. St.	15	13

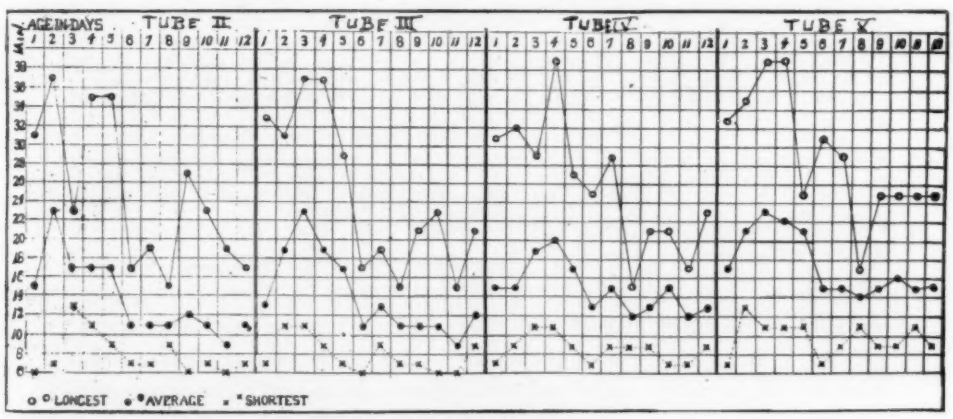

Fig. 15.—Prothrombin.

It is not within the scope of this paper to present at this time a review of our knowledge of the relation of methemoglobin, hematin, bilirubin, urobilin and urochrome. Since the work of Virchow,[25] Stadleman,[26] Küster,[27] and more recently Whipple and Hooper,[28] the identity of hematoidin and bilirubin has been generally accepted. The distinction between hematinaemia and true icterus must also be borne in mind. As concerns the lipochromes (xanthophylls and carotins), the

25. Virchow: Arch. f. Path. Anat. **1**:379, 1847.
26. Stadleman: Der Icterus, Stuttgart, 1891, pp. 3-45.
27. Küster: Ztschr. f. Physiol. Chem. 59.
28. Whipple and Hooper: J. Exper. Med. **17**:612, 1913.

work of Palmer [29] and Hess and Meyers [30] has shown that exogenous pigments are present which may produce a pseudo-icteric condition with both skin and serum manifestations.

Obviously any method which seeks to estimate the concentration of bile pigment, i.e., bilirubin in the blood, with any degree of accuracy must be specific. Numerous authors and investigators have sought to estimate the quantity of bile pigment by a comparison or dilution of the colored serum, free, of course, from blood, etc. In this country Blankenhorn [31] has made clinical studies based on this "tintometer method" in which the amount is estimated by the dilution necessary to reach the color just able to be discerned. Mülengracht [32] and his co-workers have used a similar method though somewhat more detailed in which 1 c.c. of the citrated serum is placed in a standard test tube and diluted with 0.9 per cent. sodium chlorid solution until the same tint as the standard solution, which is a 1 to 10,000 bichromate solution, is obtained. This is supposed to represent normal serum. From our present knowledge of the varieties of endogenous and exogenous chromatic elements which may occur normally and pathologically in the blood, it is obvious that these methods are open to criticism since they cannot be used to discriminate the nature of the pigment which they seek to estimate.

Ehrlich's diazo reaction was first studied in relation to the bile pigments by Pröscher.[33] In 1913, van der Bergh and Snapper [34] applied this method to the quantitative estimation of bilirubin in the blood. Since the introduction of this method and its numerous modifications a great many experimental clinical observations have been made and the test has assumed an important diagnostic use. The test has also shown differences occurring in the sera of various animals. Of recent date, Bauer and Spiegel [35] have studied the fluctuations not only in obstructive jaundice and febrile states but also under the influence of various drugs. Fiegle and Querner [36] have found the normal content increased in a wide variety of pathological conditions. These observations have not been constant in the findings by other investigators.

In comparing the diazo method with that used in our series of observations a sharp distinction is found. Thus normal adult plasmas which gave nothing but a clear white protein precipitate with the acid alcohol standard used in our determinations, nevertheless

29. Palmer: J. Biol. Chem. **17**:211, 1914.
30. Hess and Meyers: J. A. M. A. **73**:1743 (Dec. 6) 1919.
31. Blankenhorn: Arch. Int. Med. **21**:282 (March) 1918.
32. Mülengracht: Acta Med. Scandinav. **53**:827 (Jan. 14) 1921.
33. Präscher: Ztschr. f. Physiol. Chem. **29**:411, 1900.
34. van der Bergh and Snapper: Deutsch. Arch. f. klin. Med. **110**:540, 1913.
35. Bauer and Spiegel: Deutsch. Arch. f. klin. Med. **129**:17, 1919.
36. Fiegle and Querner: Ztschr. f. Exper. Med. **9**:153, 1919.

registered a positive diazo reaction, which according to v.d. Bergh, Fiegle and others, represents the normal threshold of bilirubin in human serum. Those icteric plasmas which develop the green color by our method also gave an intense red color by the diazo. This would appear at first to indicate the diazo reaction to be more delicate; but, as other workers have shown, the diazo reaction may be produced by substances other than the bile pigments.[37] In other words, the reaction is based on the chemical group which may be found in a variety of substances. Ehrlich himself at first applied the method for the estimation of unknown groups of substances in the urine. The sharp distinction between the two methods above discussed indicates, we believe, the lack of specificity in the diazo reaction. It is not within the scope of this paper to discuss the conflicting views of the relation of urobilin, urobilinogen and urochrome, but we believe the overlapping of the several tests (Russo, Weisz and Diazo) further indicates the non-specificity of the diazo reaction. The explanation of the direct and indirect diazo reaction again indicates a variety of substances which will react with Ehrlich's diazo solution.

The characteristic green color which develops on oxidation of bilirubin to biliverdin is perhaps the most specific color reaction for the bile pigments. This has been the basis for most of the well known qualitative tests such as Hammersten's and Gmelin's. It has also furnished the basis for numerous quantitative methods for determining bile pigment in both bile and blood.

Ivar Bang [38] made an excellent series of observations, especially on the icterus of the new-born in which he depended on the development of the blue-green ring at the line of contact between the serum and the oxidizing agent which consisted of 300 parts of nitric acid with 0.06 of sodium nitrate (Gmelin-Sunde technic). The speed of the development of the color and the intensity of the color gave a rough estimate of the concentration of bile pigment. A similar method was adapted by Gilbert.[39]

Fourchet [40] precipitated the proteins with chloroform and estimated the concentration of the pigment in the oxidation to the characteristic green color. Perchlorid of iron was used to hasten oxidation. This method was designed generally for qualitative work, but as Fourchet has shown, lends itself for the quantitative estimations.

Hooper and Whipple, who have made a most thorough study of the bile pigment in the bile, advocate a precipitation method for the estimation of the pigment in serum and body fluid.

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37. Herter: J. Biol. Chem. **1**:251, 1905; **4**:403, 1908.
38. Bang: Hospitalstid. **8**:637, 1915.
39. Gilbert: Presse méd. **14**:201, 1906.
40. Fourchet: Compt. rend. Soc. Biol. **80**:826, 1917.

The serum is made alkali with saturated solution of sodium bicarbonate and mixed with a 10 per cent. solution of calcium chlorid, giving a voluminous precipitate containing the bile pigments. The precipitate is thrown down and washed repeatedly with distilled water by the use of the centrifuge. The precipitate is finally dissolved in a measured amount of the nitrohydrochloric acid alcohol solution and allowed to stand at room temperature over night. The pigments are then estimated by the colorimeter.

Hedenius [41] had previously shown that qualitative bilirubin could be detected in the blood by precipitating the protein with alcohol, filtrating and acidifying the filtrate with hydrochloric acid. It is, therefore, possible that a shorter method than the precipitation method could be found for quantitative estimation. Such a method is used in the estimation of the pigment in the bile itself.

On experimentation it was found that the clear icteric plasmas presented no factors, either in the protein or the chromatic elements, which prevented the direct estimation of the pigments without precipitation. Furthermore, instead of first precipitating the proteins, as Hedenius advocated, the two processes of precipitation of the protein and oxidation of the pigment could be carried out in one.

Finally, as regards the more detailed precipitation process, it has been our observation that not only does this method involve a greater experimental error on account of the added steps, but also the calcium chlorid fails to precipitate all the pigments present, for after adding the calcium chlorid in large amounts the filtrate still gives positive tests for the presence of pigment.

Yllpö [42] used a spectrophotometric method. Undoubtedly this is one of the most reliable methods, not only for qualitative determination of the bile pigment but also for quantitative analysis. In studying the distinction of the various chromatic elements in the blood it is of infinite value. However, for a simple method, it cannot be reduced for quick routine work.

We have examined ninety cases by the method we have proposed for the examination of bilirubin in the plasma. Of these cases, seventy-two showed a positive reaction. During the course of this investigation we also noted as carefully as it was possible to do so the appearance of clinical icterus. If anyone has attempted to do this in a cosmopolitan clinic, such as we have, they will realize the difficulty of being absolutely sure of slight cases of icterus. During the period we were carrying on these observations there were often infants of as many as eight or nine different nationalities in the nursery. There is no difficulty in determining marked icterus, but we do not feel absolutely certain of the exact proportion of clinical icterus. Table 16 details the number of cases in which we were able to say that clinical icterus was present as compared with the finding of bilirubin in the plasma.

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41. Hedenius: Upsala Läkaref. Förh. **29**:541, 1893.
42. Yllpö: Ztschr. f. Kinderh. **9**:208, 1913.

This shows very definitely that toward the third day the findings in the plasma and clinical icterus correspond fairly closely. The difference is not to be taken too exactly; as already noted, it is difficult often to determine slight degrees of clinical icterus. From the third day on, the plasma and bilirubin findings and clinical icterus correspond fairly well.

Figure 16 shows graphically these same findings.

TABLE 16.—Clinical Icterus and Bilirubin in Plasma of New-Born

90 Cases Examined, 72 Positive for Bilirubin. 26 Cord Plasmas Examined, 14 Negative, 12 Positive

Days	Plasma		Clinical Icterus
	Positive	Negative	
(0-23 hours)	6	1	2
1	25	3	6
2	30	4	20
3	42	2	35
4	39	6	33
5	30	10	25
6	28	16	23
7	26	18	21

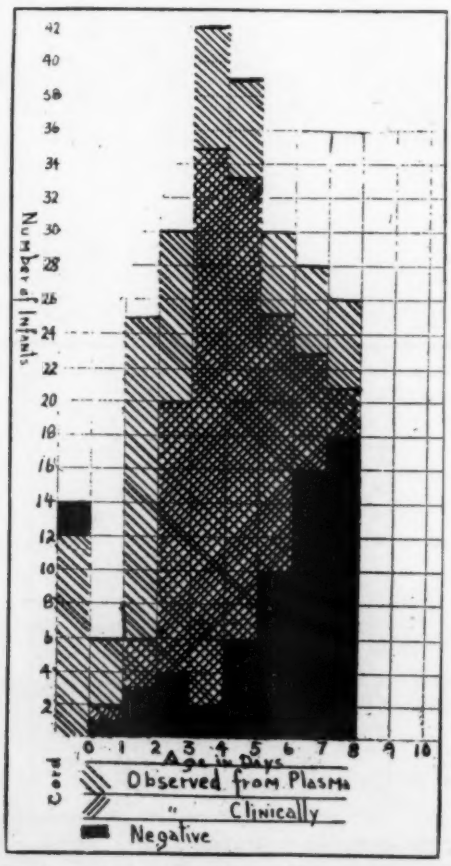

Fig. 16.—Findings in blood plasma of new-born with icterus.

Figure 17 shows an average curve of the seventy-two positive cases with bilirubin in the plasma and a typical curve of an individual case which ran above the average of the seventy-two. It might be noted here that the cases which ran a low value of bilirubin in their plasma

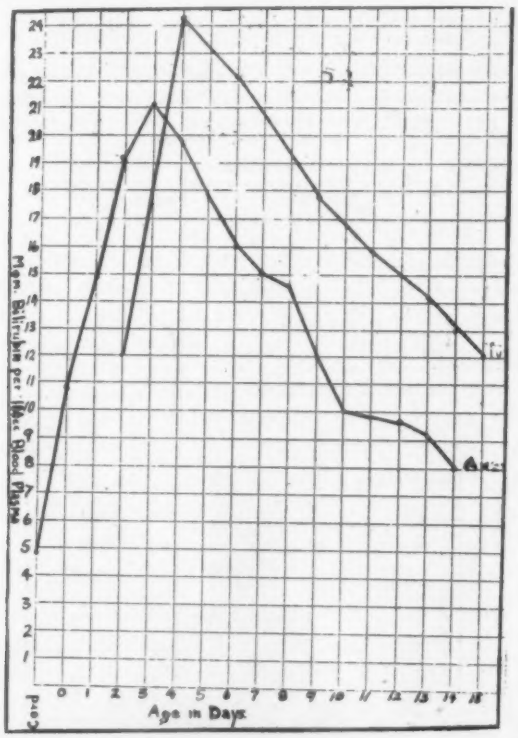

Fig. 17.—Average curve of seventy-two positive cases, and typical curve of individual case above average.

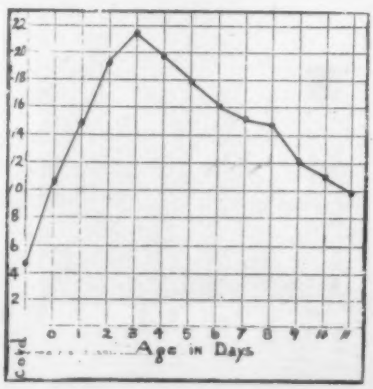

Fig. 18.—Average curve in seventy-two cases in mg. bilirubin per hundred c.c. blood plasma.

reached the maximum earlier than the high value cases which reached their maximum somewhat later than the average curve, i.e., the average curve reaches its maximum on the third day. Low value cases often reach their maximum before this day where high value cases reach it sometime after the third day and continue with high values for a longer period.

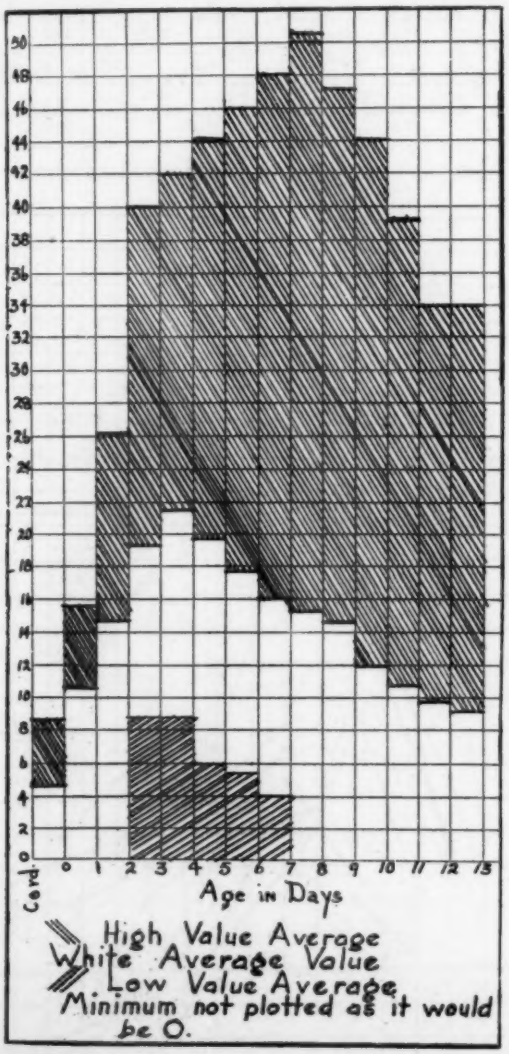

Fig. 19.—Maximum, average and low value readings in mg. bilirubin per hundred c.c. blood plasma.

Figure 18 shows a simple average curve of the seventy-two cases drawn on a somewhat different scale.

Figure 19 shows the maximum, average and low value readings in mg. bilirubin per hundred c.c. blood plasma.

TABLE 17.—Ten Cases in Which at Least Four Observations on Bilirubin in Blood Plasma Were Made

Name	Mg. Bilirubin per 100 C.c. Blood Plasma													
	Age in Days													
	Cord	0	1	2	3	4	5	6	7	8	9	10	11	12
1. Mallove...	4.7	17.4	....	....	31.3	....	20.9	....	8.7					
2. Simpson..	...	....	8.7	....	27.8	....	22.6	....	....	12.2	....	10.4	....	8.7
3. Hudekott.	...	....	19.1	....	27.0	....	....	20.9	....	19.7				
4. Bride.....	4.7	....	....	26.1	....	4.0	....	....	20.9	....	20.9	....	17.4	
5. Duarts....	...	....	....	19.1	....	....	20.9	....	19.4	....	19.1			
6. Sarris I...	...	....	....	8.7	....	12.2	....	13.9	....	....	7.0	....	5.7	
7. Sarris II..	...	....	....	10.4	....	13.9	....	19.1	....	....	5.2	....	5.2	
8. Monez.....	...	....	12.2	....	19.1	....	26.1	....	....	12.2	....	12.2	....	5.2
9. Nicholsen..	...	.. ..	13.9	....	29.6	....	17.4	....	....	15.7	....	14.2	....	12.0
10. Palmeri...	...	6.0	....	....	19.1	....	31.3	....	26.1	....	....	19.7	....	17.8

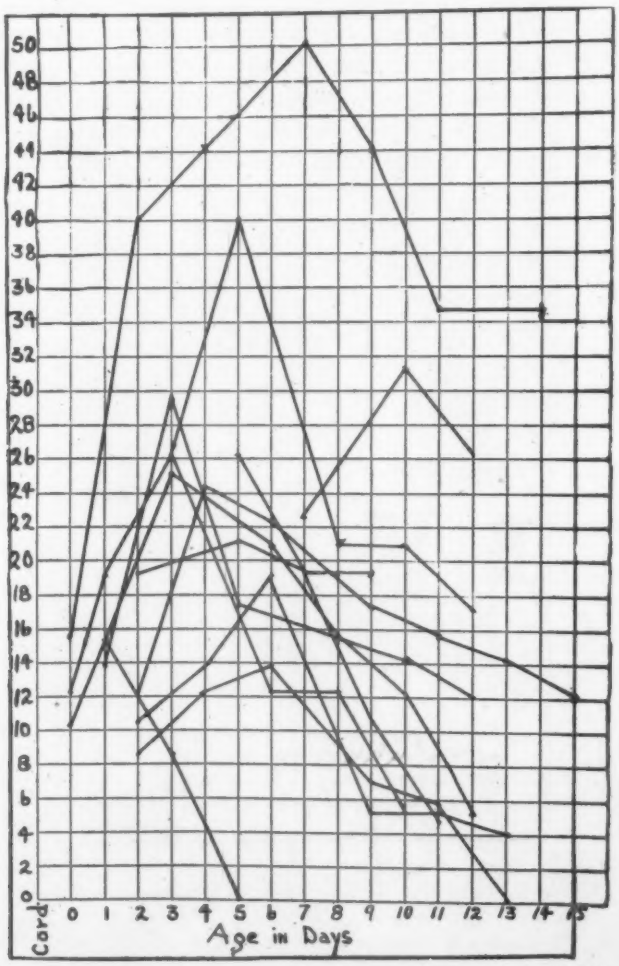

Fig. 20.—Curves of twelve cases showing variations in bilirubin in blood plasma, mg. per hundred c.c. blood plasma.

Figure 20 shows plotted curves of twelve individual cases showing the variations in bilirubin in blood plasma.

In Table 17 are shown ten cases in which at least four observations on bilirubin in blood plasma were made. This table shows very conclusively the value of this method not only as a qualitative but also as a quantitative method for estimating bilirubin in blood plasma. The + marks in the individual squares represent the day on which icterus was clinically noted. Infants Nos. 6 and 7 were twins and it can be seen how closely the bilirubin ran throughout their whole period of observation. Two cases are shown in which cord blood was also examined. In Case 1 it is very evident that in the circulating blood of the new-born there is a very much larger percentage of bilirubin than in the cord blood. In this case the blood plasma from the infant was obtained within a few hours after delivery.

At the present time we do not feel in a position to discuss the all important question as to what becomes of the red blood cells, but we do feel that this method for testing bilirubin gives us a much better means of attacking this problem. We are now engaged in carrying out a combined study of bilirubin in blood, stools and urine, together with other studies on this problem. We hope in the near future to be able to report on some of these findings.

THE CLINICAL SIGNIFICANCE OF CALCIUM CONCENTRATION IN THE SERUM OF CHILDREN AND POSSIBLE ERRORS IN ITS DETERMINATION *

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For the evaluation of calcium determinations on serum, plasma or whole blood it is necessary to know, first, whether the concentration of calcium is nearly constant and second, what factors influence this concentration. As the result of several thousand determinations in this laboratory, we believe that the calcium concentration of the serum of children is singularly constant and that the concentration is strikingly altered in only two conditions that we have met (a) tetany, (b) kidney insufficiency with or without nephritis.

In the last three years, several papers have appeared that would seem to show that the calcium concentration of the serum is not constant, that high values may be obtained even in frank tetany and that very low values may be found in the serum of children who present no evidences of tetany. This is entirely at variance with our experience. For this reason we wish to record some of our results in a variety of different diseases. We also wish to point out the more common factors that, in our experience, may lead to error in the determinations of small amounts of calcium.

The work of physiologists—Loeb,[1] Keith-Lucas,[2] Matthews,[3] Mines [4] and others—demonstrates the extreme susceptibility of the tissues, especially the nerves and muscles, to slight changes in the inorganic concentration of the tissue fluids. Especially has this been shown to be true of calcium. It would, therefore, a priori appear unlikely that marked changes in the concentration of the calcium would occur without manifest evidence of the change. An examination of available figures shows that on the whole the results of calcium determinations are remarkably constant not only for the same animal but for animals of different species. Not many series by the same experimenters are available. With cows, Meigs, Blatherwick and

* Received for publication July 1, 1921.

* From the Department of Pediatrics, The Johns Hopkins University.

1. Loeb, J.: Beitr. z. Physiol. Festschrift für Adolf Fick, 1899. Loeb, J. and Ewald, W. F.: J. Biol. Chem. **25**:377, 1916.
2. Keith-Lucas: J. Physiol. **37**:459, 1908.
3. Matthews, A. P.: Am. J. Physiol. **11**:455, 1904.
4. Mines, G. R.: J. Physiol. **37**:408, 1908.

Cary [5] found a marked constancy. Thirty of their determinations give an average calcium concentration of 10.2 mg. per hundred c.c. of plasma. The highest figure was 11.2, the lowest 9.1. We have found the average for the dog 11.1, for the sheep 10.2, for the rat 9.5. With normal adults we have found the concentration to be from 9 to 10.5 mg. and with normal children from 10 to 11 mg. per hundred c.c. serum.

CALCIUM CONCENTRATION OF SERUM IN HEALTH AND DISEASE EXPRESSED AS MG. PER 100 CC.

Normal child	10.8, 10.2, 10.2, 10.0, 11.0; placental serum: 11.1, 10.5, 10.4, 11.0, 11.0
Normal adult	9.8, 10.8, 9.4, 9.4, 10.3, 10.6, 10.8, 10.4, 9.9, 10.5, 9.8, 10.5, 10.6, 10.5, 10.3, 10.1, 10.6, 10.7
Normal adult	9.7, 10.4, 10.5, 9.8, 9.8, 9.9, 9.8, 9.6, 9.5, 9.3
Tetany	7.2 4.4, 4.0, 3.7, 5.8, 4.3, 6.3, 6.1, 5.3, 3.9, 5.6, 5.6, 7.5, 6.2, 7.4, 6.6, 5.8, 7.0, 5.0, 5.8
Tetany	5.2, 3.5, 7.0, 7.0, 6.3, 6.0, 6.7, 6.0, 5.0, 6.7, 5.5
Rickets	8.6, 9.4, 10.3, 9.8, 10.2, 10.7, 9.7, 8.5, 8.7, 10.7, 10.5, 10.5, 10.2, 10.1, 10.5, 10.8, 10.1, 9.2
Rickets	8.8, 10.0, 10.0, 10.6, 8.6, 8.3, 8.0, 9.8, 7.8, 9.3, 9.7, 8.0, 9.6, 11.1, 7.8, 8.2, 9.7, 9.0, 10.1 8.1
Rickets	8.7, 10.0, 11.0, 10.6, 8.2, 9.4, 10.1, 9.1, 9.2, 9.0, 9.3, 9.0, 8.3, 11.0, 11.0, 11.0, 8.3, 8.3
Pneumonia	9.7, 10.5, 9.0, 10.1, 10.4, 11.9, 10.5, 9.4, 8.0, 9.6, 9.4, 11.0, 10.6
Scurvy	9.8, 10.3, 10.8, 10.6, 10.0, 10.1, 10.9
Epilepsy and mental deficiency	10.7, 11.0, 10.5, 9.0, 11.5, 11.1
Nephritis	6.0, 7.7, 7.5, 10.5, 8.5, 8.3, 5.3, 9.0, 8.4, 10.5, 11.0
Renal insufficiency with diarrhea	7.4, 4.8 (soda), 5.9 (soda), 7.0, 8.7
Burns	11.0, 8.2, 9.0, 9.0
Syphilis	10.4, 9.5, 10.5
Acute rhinopharyngitis	9.6, 10.6
Osteogenesis imperfecta	11.1, 10.4, 12.1, 11.0
Chorea	9.3, 10.1
Purpura	8.2, 9.4
Chronic endocarditis	9.4, 10.0
Obesity (adult)	11.0
Diabetes	10.6
Pulmonary tuberculosis	10.0, 9.3
Diphtheria	10.6

The accompanying table contains the results of a number of calcium determinations made in this laboratory on the serum of normal adults, normal children and children suffering from various diseases. The children with rickets or tetany were all under three years of age, some of the others were older. As previously stated, the concentration of calcium in the serum of normal adults is usually lower than that of normal children. Physiological conditions such as diet, exercise, etc., seem to exert little if any influence on the calcium of the serum.

In a series of thirty-one cases of active tetany the calcium concentration in the serum varied from 3.7 to 7.4 mg. per hundred c.c. serum, the average being 5.8 mg. Fifty-six cases of rickets showed an average concentration of serum calcium of 8.6 mg. per hundred c.c. The lowest value found was 8.0 while the highest was 11.1 mg. For reasons that we have considered fully elsewhere, we believe that the low concentration of calcium not infrequently found

5. Meigs, E. B., Blatherwick, N. R., and Cary, C. A.: J. Biol. Chem. **37**:1, 1919.

with rickets depends not on the rickets per se. but on an associated latent tetany.

In nephritis or in renal insufficiency not associated with nephritis (polycystic kidney, burns, dehydration with severe diarrhea, etc.) the concentration of calcium was low in twelve out of twenty cases. Some of these values, especially when infants had been treated with soda, are comparable to the values found in active infantile tetany. In several of the children treated with soda symptoms of tetany developed. In no other of the clinical conditions studied did we find any significant change in the concentration of calcium in the serum.

Our figures do not confirm those published by Denis and Talbot.[6] These authors found a very low calcium content of the serum with respiratory infections, especially pneumonia, but on the other hand they found exceedingly high figures with the same infections. Thus, in pneumonia their variation was from 12.1 to less than 2 mg. per hundred c.c. In a series of thirteen cases of pneumonia, three of which were fatal, we found essentially normal figures in all instances but one. This one child, suffering from rickets, had a calcium content of 8.0. From our own experience with calcium determinations in pneumonia and other diseases it is impossible for us to understand the figures published by Denis and Talbot. With diseases such as epilepsy, chorea, mental deficiency and mild nutritional disorders which we have never found associated with any calcium disturbance, they report variations from 1.0 to 13.5 mg. per hundred c.c.

Nor do our findings confirm those of Hess and Killian[7] in regard to infantile scurvy. These authors reported calcium determinations on the plasma of seven children with scurvy, the concentration in all cases being low, from 3.4 to 7.2 mg. Later Hess states that subsequent determinations "have generally yielded normal results." We have had a series of seven cases of scurvy, all in the active stage. The results have been normal with every determination (9.8-10.9, average 10.3).

We are quite unable to understand the findings of Brown, MacLachlan and Simpson,[8] who state that they found an average of 9.5 mg. calcium per hundred c.c. of *whole blood* in normal infants. This figure is only 10 per cent. less than the average for the *serum* of normal infants. If these figures are correct, one would have to assume practically the *same* concentration of calcium in the corpuscles and in the serum. We have consistently failed to find any calcium in normal human corpuscles and even those who assert that calcium may be present in the corpuscles recognize that the amount is very small.

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6. Denis, W., and Talbot, F. B.: Am. J. Dis. Child. **21**:29 (Jan.) 1921.
7. Hess, A. F., and Killian, J. A.: Proc. Soc. Exper. Biol. & Med., **16**:43, 1918.
8. Brown, A., MacLachlan, I. F., and Simpson, R.: Am. J. Dis. Child. **19**:413 (May) 1920.

Stheeman [9] in the course of a study of the electrical excitability of the nerves of children found no consistent relationship between the concentration of the calcium in the serum and the irritability of the nerves. Our experience has taught us that there is a very definite relationship.

We feel that it is proper, since our results have been very consistent for a number of years, with a great many determinations, to call attention to the sources of error that may be encountered in determining calcium with small quantities of plasma, serum or blood. With all micro methods the permissible error is greater than with methods in which large amounts of material are used. It is only through extreme care that errors are avoided in micro methods. Only reagents that have been proven to be free from calcium should be employed. Nitric acid, which is used in the method of wet ashing of blood, may, at times, contain calcium in measurable amounts. Trichloracetic acid, a frequently used deproteinizing agent, may likewise contain calcium. Even distilled water may contain traces of calcium. It is, therefore, advisable to use as few reagents as possible. Lyman [10] called attention to the presence of calcium in filter paper. When trichloracetic acid is used for the removal of protein from serum or whole blood and the coagulum separated by filtration through a high grade acid-washed filter paper, we have found that the filtrate may remove from the filter paper a quantity of calcium sufficient to give an error of 75 per cent. in the determination of this element. Thro and Ehn [11] have had a similar experience. Since it is impossible to tell what samples of filter paper contain calcium we have dispensed with filtrations in all calcium determinations in serum and whole blood.

While the presence of calcium as a contamination may account for occasional high results, the low figures more frequently found, require a different explanation. Kohlrausch [12] and Richards and his collaborators [13] have found that calcium oxalate is not wholly insoluble in water, so that if the precipitate is washed a loss of calcium may occur. This may be reduced to a minimum by washing in such a way that the precipitate is in contact with a dilute solution of ammonium oxalate until the last stage of the washing. We have controlled our own determinations by using a method in which washing is not required.

The precipitation of calcium as the oxalate from serum directly or from solutions of serum ash or serum which has been freed of its

9. Stheeman, H. A.: Jahrb. f. Kinderh. **94**: 1921.
10. Lyman, H.: J. Biol. Chem. **29**:169, 1917.
11. Thro, W. C., and Ehn, N.: Proc. Soc. Exper. Biol. & Med. **18**:189, 1921.
12. Kohlrausch: Quoted from Seidell—Solubilities of Inorganic and Organic Compounds, Ed. 2, D. Van Nostrand Co, New York, p. 209.
13. Richards, T. W., McCaffrey, C. F., and Bisbee, H.: Proc. Acad. of Arts and Sciences **36**:375, 1901.

protein must be accomplished in the presence of both magnesium and phosphates. When solutions of serum ash are rendered alkaline with ammonia a fluffy precipitate of calcium phosphate forms. This must be completely redissolved before the addition of the oxalate. The concentration of either magnesium or phosphate in unashed serum or plasma is small, except in certain pathological conditions.[14] Richards and his collaborators have determined the conditions under which a quantitative separation of calcium from magnesium may be accomplished. The concentration of magnesium in serum and plasma is so small that it cannot be considered a serious source of error.

McCrudden [15] showed that unless the solution from which calcium is precipitated is sufficiently acid, the calcium will be precipitated in part as the phosphate. If a precipitation of calcium phosphate occurs titration with permanganate will yield low results. We have found the optimum p_H of the fluid for the quantitative precipitation of calcium as the oxalate to be from 5.2 to 6.2. As the alkalinity increases the amount of calcium that is precipitated as the phosphate also increases. The error in determination thus caused may be very large. The addition of sodium citrate to blood increases the alkalinity of the plasma and may result in the precipitation of part of the calcium as the phosphate. Plass [16] states that this may be avoided by using sodium citrate that has been neutralized to litmus by the addition of citric acid. The amount of fluid in which precipitation is accomplished and the length of time allowed for precipitation are also important factors.

The use of hydrochloric acid in an analysis in which the final determination is a permanganate titration should be avoided since hydrochloric acid is oxidized by permanganate to chlorine. According to the method used this may yield high or low results. With reasonable care there is no difficulty with permanganate titration in reading the end point.

CONCLUSIONS

A large number of calcium determinations on the serum of normal children shows a marked constancy in the concentration of this element.

In only two conditions commonly met with, tetany and renal insufficiency, is the concentration of calcium markedly reduced. In no condition has it been found increased above normal limits.

There are many possibilities of error in methods for the determination of small amounts of calcium. Some of the more common of these are discussed.

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14. Marriott, W. McK., and Howland, J.: Arch. Int. Med. **18**:708 (Nov.) 1916.
15. McCrudden, F. H.: J. Biol. Chem. **7**:83, 1909.
16. Personal communication.

GENITAL TUBERCULOSIS IN MALE CHILDREN *

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Although tuberculosis in one form or another is common in children, this disease seems to attack the genital and urinary organs comparatively rarely, a point on which most investigators are in agreement. There is reason to believe, however, that tuberculosis of the genital tract is not so uncommon as has been supposed, the difficulty lying in the fact that it may be mild, that a spontaneous cure may be effected, and that because of these facts the condition may either be overlooked or ascribed to an infection by organisms other than the tubercle bacillus. Be that as it may, large numbers of cases have not been reported.

Poissonnier,[1] in a very complete review of the subject of infantile genital tuberculosis, collected ninety-one cases from various sources, the greatest number, forty-four, having been observed by Broca among 46,000 juvenile hospital inmates. Since this time various writers have reported from one to three or four cases each, so that it would not appear that advances in diagnostic acumen were reaping a much richer harvest.

It is not, therefore, surprising to find that the Massachusetts General Hospital has shared in this comparative paucity of material. A search of the records from 1872 to date reveals a total of eleven such cases, among 401 admissions (including reentries) for this condition, a percentage of 2.74.

Various statements are made to the effect that the disease affects particularly very young children, decreasing in frequency as puberty approaches. Poissonnier, among eighty-nine cases, found forty-two occurring before the second year, forty-seven between the second and fifteenth year. Among our eleven cases the youngest child was 9 months old, the other children being, respectively, 18 months, 2 years, 2½ years, 5 years, 6 years, 6½ years, 7 years, 8 years, 12 years and 14 years of age. That the nursing infant is not spared is shown by two such patients reported by Swoboda,[2] and we are even informed that a case of this disease in a fetus at term has been observed by Drechsfeld (quoted by Poissonnier [1]).

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* From the Genito-Urinary and Children's Medical Departments of the Massachusetts General Hospital.

1. Poissonnier: Gaz. des hôp., 1907, pp. 375-380.
2. Kirmisson: Rev. gen. de clin. et de Therap. **32**:641-644, 1918.

In our series of eleven cases both epididymes were found to be involved at the time of entrance in one case (an infant aged 9 months); in another case (a boy aged 8 years) the second side became involved twenty-seven months after removal of the epididymis first affected, while in a third case the involvement of the second side took place within three months after excision of its tuberculous mate (orchidectomy). These facts seem to be in accord with the views of other writers, especially Kirmission [2] and Poissonnier,[1] the latter having collected only seven bilateral infections out of forty-two cases. Bilaterality of the disease in children is therefore far less common than in adults. My own statistics, published some years ago,[3] showed that at the time of entrance to the hospital both sides were involved in 29.3 per cent. of 150 adult cases, while König found bilateral lesions in 75 per cent., and von Brun found both sides involved in 38 per cent. At the Tübingen clinic, 29 per cent. of 111 cases were found to be bilateral.

The remaining eight cases in our series showed about an equal distribution of the disease between the left and right sides.

Kirmission [2] states that in adults the testicle itself is often intact, whereas in children this organ is frequently diseased. On the other hand, Vignard and Thévenot [4] assert that the testicle and epididymis are attacked simultaneously, a view which is shared by Poissonnier,[1] who states that the burden of infection is shared equally by these organs.

Our own experience shows that in only two instances was the testis itself definitely tuberculous, but it should be stated that the pathologic reports, more particularly those of an earlier day, are very vague as to the differentiation between testicle and epididymis. I believe that there is much less frequently an involvement of the infantile than of the adult testicle, and that in most cases it is no more necessary to remove the testicle of a child than that of an adult. Indeed, it may be said that the removal of this organ from patients under the age of puberty should be done only as a last resort, with much less impunity than in the adult, owing to the demand made on its invaluable internal secretion as puberty approaches.

While the formation of a scrotal abscess with spontaneous rupture and the establishment of a fistula may occur in children as in adults, it certainly is a far less common phenomenon. I have already showed that such an event takes place in over 76 per cent. of adult cases, whereas in the children under discussion scrotal fistula was found only three times.

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3. Barney: Mod. Urol. **1**:489-533.
4. Vignard and Thévenot: Ann. d. méd. et de chir. Enf. **15**:561, 1911.

While it is unfortunate that our records definitely state that the vas deferens was involved in only two instances, there is no reason to believe that the seminal duct of children is less frequently invaded by tuberculosis than that of the adult. In the latter, such an involvement is found in the great majority of cases, and according to Poissonnier [1] the vas was definitely tuberculous in eighteen out of twenty-eight children in whom this point was noted.

It is unfortunate also that in so few cases is not only the condition of the vas deferens, but also that of the prostate and seminal vesicles recorded. Since so much hinges on the knowledge of the condition of the latter organs not only from the scientific, but also from the prognostic viewpoint, it is disappointing to find that no mention whatever is made of these organs by many observers. While it is true that in children rectal examination is not easy and may be productive of pain, the facts can readily be ascertained if and when the child is anesthetized for operation.

The records of our cases compare favorably with others in this respect, definite statements as to the condition of the prostate or seminal vesicles or both having been made in six cases, twice at necropsy and in the other four either at or after operation. Of the latter, in a boy aged 8 years, the seminal vesicles were recorded as "palpable" and "very tender" before operation, whereas over two years later, tuberculosis of the second epididymis and of one kidney having meantime intervened, the record states that the "prostate is normal and the vesicles are not felt." In a boy aged 14, with unilateral epididymitis of short duration it is recorded that "nothing is felt in the prostate," and in a child 5 years of age, with a tuberculous epididymitis of over two years' duration on the one side, and of from three to four months' standing on the other, we are told that the rectal examination was "negative." Finally, a boy aged 7, having a unilateral orchidectomy in 1905, was examined at the hospital in 1911 and was found to have a normal prostate and seminal vesicles and the remaining testis and epididymis free from disease.

Although these cases will be dwelt on later at greater length I may say that in two cases seen at necropsy, one with bilateral disease and unoperated, showed an extensive involvement of the seminal vesicles and a slight and early invasion of the prostate, whereas the second patient having had an operation on one tuberculous testicle died, twenty-six days later, and was found to have a normal prostate and seminal vesicles. Thus five out of six patients are known to have had no demonstrable lesion of the prostate or seminal vesicles.

Kantorowicz, in fifty-seven cases of epididymal tuberculosis in children, found the prostate involved but twice. Broca, who examined all of his forty-four cases, is reported never to have found a lesion

of the prostate or vesicle. In support of these views Kirmission states that it is much more rare than in the adult to find lesions of the prostate and seminal vesicles in children. On the other hand, Vignard and Thévenot state that in these young children "the vesicles and prostate are often the seat of indurated nodules or of suppuration.' There seems, therefore, to be a difference of opinion but I am inclined to believe that the question of prostatic and vesicular tuberculosis could be settled definitely if careful routine examinations were made of these organs. It is worthy of note, however, that in a careful review of the literature of this subject no mention whatever is made of any instances of tuberculosis of the prostate or seminal vesicles being noted before the onset of epididymitis.

From what has been stated, we may conclude that while tuberculosis of the prostate and vesicles may, and does, at times occur in children, it is certainly a far different situation than that which exists in the adult. In previous investigations of this question I found that in the adult these organs were definitely involved in seventy-six out of 101 cases, and I stated at that time that "the combined experience of all observers shows that prostatic and vesicular tuberculosis is most frequent during the time of the greatest activity of these organs, i. e., from about the twentieth to the fortieth years."

The comparative freedom from disease enjoyed by the infantile prostate may well account for the fact that I have nowhere seen it stated in the literature, nor has it been our own experience that bladder irritability or abnormal urinary constituents played a great part in the symptomatology of this disease in children. It also seems to be true that while in the adult renal tuberculosis sooner or later intervenes in a considerable number, constituting true "genito-urinary tuberculosis," this combination attacks the child with comparative rarity. In our eleven cases there was but one, a boy aged 8, with renal tuberculosis, a complication which was discovered shortly after his first appearance at the hospital. In two cases the urine contained a few leukocytes and the slightest possible trace of albumin. Other than this there is no evidence in these cases of any damage to the urinary tract either before or after operation.

It is generally recognized that while the primary focus of tuberculosis in the urinary tract is in the kidney and while there are those who believe that in the genital tract this focus lies in the epididymis, it is equally certain that both foci are really secondary to a third. The statistics of all writers agree that this focus is generally in the lung or bronchial lymph nodes, and while it cannot always be demonstrated by the ordinary bedside methods of chest examination, the roentgen ray will reveal it in almost every instance.

In the eleven cases here presented there was evidence of lung involvement in five, of tuberculous peritonitis in two, of chronic otitis media (probably tuberculous) in two, of bone tuberculosis in two, of cervical adenitis in one. In three cases no definite focus was established. It is obvious, therefore, that while disease of the lungs heads the list, it is equally clear that in a few there was evidence of active tuberculosis in more than one focus outside of the genital tract.

In view of these findings it is not surprising to observe that eight of the eleven patients were in bad condition when admitted to the hospital, loss of weight, anemia, rickets and malnutrition being predominant features. Such a large percentage of poor risks is in distinct contrast to the picture often presented by the adult suffering from a similar involvement of the genital tract. As often as not he is well nourished and superficially sound in appearance, the foci of disease being discovered sometimes only after the most painstaking search.

The surgical treatment of these cases consisted of orchidectomy in seven, and epididymectomy in two (of which one was bilateral). Of the remaining two one patient was unoperated and died of meningitis shortly after coming to the hospital, and the other was so occupied with the relief of his tuberculous peritonitis, and later of his empyema, that the treatment of his epididymis was left for another occasion.

A pathologic examination of the tissue removed was made in eight cases, all of which showed the characteristic lesions of tuberculosis. It is of interest to note that in six of these cases the presence of caseation indicated that the process was of long duration, much longer than would be supposed from the history obtained.

The outcome, more or less complete, is known in ten of these cases.

One child 2 years old, having left orchidectomy, was brought back the following year with a relapse on the opposite side. No further facts are known.

A boy, aged 6½ years, was seen a year after the removal of the right testicle and epididymis. His general and local condition were satisfactory as was that of the opposite testicle. The urine was negative.

Another boy, 12 years of age, returned a year after unilateral orchidectomy in excellent general condition.

A fourth child, 5 years of age, was seen two and a half years after bilateral epididymectomy. The note reads, "General condition excellent. Good appetite and has gained weight. Urine clean, negative. Has diurnal frequency. Scars not adherent to testes; no induration." This case is interesting as at the time of the operation the child had a bad interstitial keratitis and many râles in the lungs.

Another boy, 7 years old, was in very good general condition at the time of his unilaterel orchidectomy. He returned six years later

when the following observation was made: "General condition good. Has gained in weight. No urinary symptoms. Right testis and epididymis normal. Prostate and vesicles normal. No fistula. Urine negative."

A sickly boy of 14 entered with tuberculous peritonitis, an old Pott's disease of the spine, and a unilateral epididymitis. After expectant treatment and being improved he was discharged. Nearly a year later he was readmitted for a cold abscess of the abdominal wall which was incised and drained. He was discharged in due time only to return three months later with empyema which was relieved by tapping. The condition of the epididymis had meantime grown somewhat worse but there was "nothing felt in the prostate." A few days later he was operated on for empyema, an operation from which he recovered fairly well. During the next three years he was a frequent visitor to the outpatient department, remaining in very fair condition. Since no note is made as to the condition of the genital tract it may be inferred that the disease was at least quiescent.

A boy, aged 8, came in for relief of a scrotal abscess. Left epididymectomy was performed. A positive guinea-pig test of the bladder urine showed the presence of renal tuberculosis. Nearly three years later, the boy was readmitted and a left nephrectomy was performed. The opposite epididymis had meantime become involved.

During the next year or two he returned to the outpatient clinic and during this time he developed a tuberculous stricture of the bulbous urethra, which required frequent dilatation. It may be said also that he was meanwhile being given tuberculin and kept under good hygienic surroundings, yet in spite of these measures his decline was slow but steady. I have reason to believe he is now dead.

Three of these children are known to have died, a mortality of over 27 per cent., but a figure which does not by any means give the facts, as none have been followed a sufficient length of time. As I have already pointed out "my experience warrants the conclusion that until at least ten years have elapsed after operation, no patient can be said to be cured of genital tuberculosis." Further and more recent observations have only served to confirm the truth of this statement.

A child, aged 1½ years, had unilateral orchidectomy. In addition to the genital tuberculosis, he had a chronic otitis media, cervical adenitis, and a tuberculous process of the ankle; altogether he was in poor shape. The wound had not healed at the time of his discharge, six weeks after operation. There is a note in the record to the effect that the patient died about four years later. The cause of death is not stated.

A necropsy was obtained in two cases.

REPORT OF CASES

CASE 1.—*History.*—A boy, aged 6 years, with a family history of tuberculosis, is said to have had "meningitis" four years before entrance. For some time he had been in poor health, with cough, fever, abdominal pain and vomiting, loss of weight, and for two months he had had a swollen scrotum. There were no urinary symptoms.

Physical Examination.—Patient was a pale, sickly child, with definite tuberculosis of the left lung, and evidence of tuberculous peritonitis. The left scrotum was occupied by a large, hard, nodular mass. Orchidectomy was performed under ether. The pathologist stated that the "testis was studded with tubercles and the cord was found in the same condition." After a stormy convalescence, the patient developed tuberculous meningitis and died on the twenty-sixth day after operation.

Fig. 1.—Entire genital tract of child 9 months of age. The middle portions of the vasa are of normal size. The seminal vesicles are greatly distended.

Necropsy.—Tuberculosis of the bronchial lymph glands, miliary tuberculosis of the lungs, liver, spleen and kidneys, tuberculous peritonitis, tuberculous meningitis, a solitary tubercle of the cerebellum, left otitis media, chronic pleuritis. The bladder, prostate, seminal vesicles and right testicle were "not remarkable."

CASE 2.—*History.*—A baby, aged 9 months, was seen December, 1920, in the Children's Medical Service at the Massachusetts General Hospital in consultation with Dr. F. B. Talbot. The family history was negative. He was well up to the seventh month when he developed a middle ear abscess followed by mastoiditis requiring radical operation. This process never entirely healed. Two months before coming to the hospital he developed a swelling of the left scrotum. At the time of entrance the child looked emaciated and sick. There was a chronic discharge from the ear and subsequent roentgen-ray examinations of the chest showed a glandular and peribronchial process, probably tuber-

culous. The urine always contained albumin and a few leukocytes. Search for the tubercle bacillus was negative. There was moderate leukocytosis. Von Pirquet test was strongly positive. Both epididymes were swollen, especially the left, and firm and nodular. Scrotal skin adherent. Moderate tenderness.

Some days after entrance the child developed bronchitis and tuberculous meningitis to which it succumbed forty-six days after entrance.

Necropsy Report.—Tuberculous ulcers of the intestines, of the mesenteric, retroperitoneal, and bronchial lymph nodes, and miliary tuberculosis of the lungs, liver, spleen and kidneys. There was also meningeal tuberculosis.

Fig. 2.—Enlarged view of prostate, vesicles and ampullae. Prostate is of normal size; vesicles and ampullae, especially the left, are enormously distended.

Fig. 3.—Testes and epididymes. Testes are entirely normal; epididymes are completely caseous and much enlarged.

The entire genital tract was dissected out by the pathologist, Dr. Oscar Richardson, to whom my thanks are due, as may be seen in Figures 1, 2 and 3.

The epididymes are much enlarged and caseous throughout. The testicles show no macroscopic changes. The vasa deferentia are thickened to the size of a goose quill at the outer third of their length, whereas in the middle third there is no gross abnormality. The ampullae are enormously distended. Both seminal vesicles, especially the left, are several times their normal size and on section are extensively caseous, there being but little normal tissue left. The

prostate is of essentially normal size and appearance. On section it shows caseous areas within and immediately around the ejaculatory ducts throughout their entire course through the prostate. In one lateral lobe there is an ill-defined, partly caseous area occupying about one-fourth of the lobe.

Microscopic study of these organs was made by Dr. James Homer Wright, to whom I express my sincere thanks.

Prostate: The opaque areas in the region of the ducts are typical ejaculatory tuberculosis, with much necrosis. The opaque area occupying about one quarter of the left lateral lobe shows small foci of necrosis in a cell-rich inflammatory tissue, in which are many epithelioid cells and some fibrous tissue. In the other lobe there are some small focal accumulations of epithelioid cells undergoing necrosis and constituting apparently recent tubercles. The microscopic appearance suggests a more recent process than in the ejaculatory ducts and in the epididymes (Figs. 4 and 5).

Epididymes: The enlarged caseous epididymes show appearances of tuberculosis of more long standing character. But little of the original tissue remains. As indicated by the gross appearance the tissue is largely necrotic, but actively growing tubercles are present at the margin of the necrotic mass in the fibrous inflammatory tissue. The testicles are normal.

Spermatic Cord: Sections from the median portion show appearance of tuberculosis.

Certain writers such as Vignard and Thévenot [4] have attempted to divide genital tuberculosis into two groups, namely those in which the affection is essentially primary in the genital tract, and those in which this involvement is a secondary feature to visceral tuberculosis. I do not believe that this grouping is desirable or accurate as those placed in the second group are merely the more advanced stages of the first. The ultimate results of these cases would appear to confirm this view.

There seems also to be a considerable division of opinion as to treatment of this condition. While practically all are agreed that general hygienic measures, to which the use of tuberculin is added by some, are essential to the prospects of cure, the handling of the local disease is a bone of contention. Jullien, in 1889, and Rocher, in 1904, expressed strong objections to surgical intervention or only such as was absolutely necessary. On the other hand Felizet, in 1899, and Phocas, in 1898, advocated more radical measures involving castration.

More recent writers, such as Poissonnier, think it unwise to use anything more radical than the curette when the case is complicated by tuberculosis of other parts. Vignard and Thévenot think that those cases occurring in the first group in which the genital infection is a primary isolated manifestation, should be submitted only to the most conservative measures, whereas those in the second, with active lesions elsewhere, should have castration. They finally ask, however, if the difference between the primary and secondary forms is as great as one thinks.

As to the ultimate results of these cases in children Jullien says that he has lost none of twenty cases. Hutinel and Deschamps, on the

Fig. 4 (Upper).—Left lobe of prostate, showing tuberculosis of the ejaculatory duct. The opaque area in upper portion shows foci of necrosis.

Fig. 5 (Lower).—Right lobe of prostate showing a few recent tubercles.

contrary, have lost six out of nine cases. Vignard and Thévenot lost two out of their three patients, one dying six months, the other two months after the onset of the local infection. Most of the statistics on this point which I have seen are of little value, however, as there is no evidence that the patients have been followed for a sufficient length of time, an objection which applies to the cases I have here recorded. I have already expressed the opinion that until at least ten years have elapsed after operation, no patient can be said to be cured of genital tuberculosis.

Since the question of the primary focus and of the means by which the disease spreads from this focus to other organs is still open to question, I think that the views of two authoritative students of the problem, although at variance, are none the less interesting.

After many years of study of this question and basing his opinion on the results of 200 necropsies of those dying with genital or genito-urinary tuberculosis Simmonds [5] comes to the following conclusions:

1. The primary focus in the genital tract proceeds from the prostate in one-half the cases, while in one-quarter of the cases it arises in the seminal vesicle or in the epididymis. This statement is based on forty necropsies in which he found the prostate alone involved in twenty, the seminal vesicles alone in ten, and the epididymis alone in ten.

2. From these centers the process can proceed in the direction of the testicle or away from it.

3. The process of healing seldom occurs in the prostate and seminal vesicles, while in the hilum of the testicle cicatrization is frequent.

4. The prognosis of male genital tuberculosis, particularly that of the seminal vesicle, is bad. The chief danger lies in the onset of tuberculous meningitis or of miliary tuberculosis. One third of the cases of genital tuberculosis in men die of this complication.

5. In the necropsies in twenty cases of men having previous castration for genital tuberculosis there was always found an involvement of the intra-abdominal portion of the genital system. Healing was not observed in these cases.

6. Kraemer: Beitr. z. Klin. d. Tub. **33**:259, 1914; **35**:119, 1915; Deutsch. med. Wchnschr. **46**:435, 1920.

Simmonds himself acknowledges that there is a marked discrepancy between the clinical results and anatomic findings, but is unable to explain it. On the strength of his pathologic experience he advocates vesiculectomy, but feels that prostatectomy for tuberculosis is a dangerous procedure.

Kraemer,[6] an equally authoritative and ardent student of the question, disagrees with Simmonds, basing his views on clinical as well as on anatomic findings. He believes that the spread of tuber-

5. Simmonds: Beitr. z. Klin. d. Tub. **33**:35, 1914; **34**:173, 1915.

6. Kraemer: Beitr. z. Klin. d. Tub. **33**:259, 1914; **35**:119, 1915; Deutsch. med. Wchnschr. **46**:435, 1920.

culosis with the natural secretory current within the male genital system holds true, and finds not only no evidence against this view in Simmonds' work, but even every evidence to support it. Regardless of what Simmonds found at necropsy, Kraemer believes that the undeniable fact that 80 per cent. of permanent cures of genital tuberculosis in the male reported by surgeons in general controverts any statement. If, he argues, the prostate is the primary focus, how can the removal of a secondary focus in the testicle produce this result. The difficulty lies in the fact that the patients seen by Simmonds had the misfortune to die and were among those the clinician failed to cure. Furthermore, as Simmonds himself acknowledges, the deaths are largely from miliary tuberculosis and meningitis, and these lesions might just as well be produced by the focus which gave rise to the genital infection, as by the genital infection itself.

Simmonds, in a subsequent article, refutes all of Kraemer's statements. He claims that one finds recent tuberculous changes in the epididymis along with far advanced changes in the prostate and vesicles. Also he thinks if the epididymis is the primary focus of genital tuberculosis, then isolated tuberculosis of the testicle should be met with more than isolated tuberculosis of the seminal vesicles and prostate, but this is not the case. Simmonds again acknowledges the great discrepancy between anatomical and clinical findings but again is unable to explain it. He concludes by remarking that whoever wishes to argue in favor of simple castration for genital tuberculosis should collect cases in which individuals so operated on will show no tuberculous changes after death.

To these arguments Kraemer replies with renewed vigor. He says it is useless to deny the report of many able surgeons that a clinical cure is effected in 80 per cent. of cases and to place against this indisputable evidence only 20 cases which were seen at necropsy. He thinks that the very fact that Simmonds himself cannot explain his findings is proof that his argument cannot hold water.

More recently Young,[7] in a remarkably able and complete article, comes out with the assertion that genital tuberculosis arises not in the epididymis, nor in the prostate, but in the seminal vesicle.

Out of this welter of disagreement what are we to believe? In the first place, it is clear that those who have studied this question experimentally, clinically and anatomically are still uncertain as to the actual facts. In an experience of several years in the study of this problem I have found the most perplexing and conflicting evidence both clinical and anatomic. While I still believe that the great weight of evidence favors the origin of genital tuberculosis in the epididymis,

7. Young: Tr. Am. Assn. G.-U. Surg. **13**:173, 1920.

I am willing to believe that in certain instances the primary focus may lie in the prostate, possibly even in the seminal vesicle. It is impossible to take up and discuss seriatim and ad infinitum the argument of the various proponents of each point or origin, more especially those of Young. One can only make generalizations.

If genital tuberculosis arises in the prostate or even in the seminal vesical why is it that this disease is practically unknown clinically or postmortem? It is only reasonable to suppose that tuberculosis of either of these organs would produce symptoms for which relief would be sought and treatment given. Furthermore, prolonged observation of such cases would undoubtedly reveal the actual condition sooner or later. It is also true that in not a few instances necropsy has shown tuberculosis of almost every organ in the body, the prostate or seminal vesicles alone being spared.

If the prostate or seminal vesicle is the starting point of epididymal tuberculosis why is it that the removal of the epididymis has such a salutary effect on the other organs of the genital tract? I have followed a good many cases for a good many years both before and after operation. While there have been occasional exceptions, as is to be expected, the prostate and vesicles which were nodular, indurated and enlarged before epididymectomy have eventually returned to an essentially normal condition. It is probably true that while the microscope would still show evidence of tuberculosis in some, it is equally true that fibrous changes eventually occur and a clinical and permanent cure is established. This statement is not a case of the wish being father to the thought, but is an actual statement of fact.

In this connection one cannot avoid touching on the subject of treatment. The results just mentioned have occurred after epididymectomy or orchidectomy, yet there are those, of whom Young is the most recent and ardent, who urge the desirability of and the necessity for the removal of the entire genital tract. While this operation certainly gives free scope to the skill, patience and ingenuity of the surgeon, it apparently does not seem to afford an equal amount of satisfaction to the patient and after all, surgery should have this as one of its objects. If the prostate will quiet down spontaneously by a simple procedure why remove the prostate? Also is it not against all surgical principles for a primary focus to subside after the removal of a secondary focus. Why not remove the entire ureter and the bladder as well as the kidney in treating renal tuberculosis? The procedure would be quite as logical. Or why not cure cancer of the breast by dissecting out the glands in the axilla?

If the primary focus lies in the prostate or seminal vesicle why are these organs less frequently attacked in children than in adults?

Yet this seems to be the fact judging from my own experience and from the reports in the literature.

The radical operation of removing the entire genital tract is thought to reduce the immediate as well as the ultimate mortality. Does it do so? In the past ten years more than 100 cases of genital tuberculosis have been admitted to the genito-urinary service of the Massachusetts General Hospital. All but a few patients have had either epididymectomy or orchidectomy performed. There has been one death in the hospital in this time, and this patient was not operated on. It would not appear, therefore, that conservative surgery involved a high death rate. It is true that the ultimate deaths from tuberculosis are high and that at least ten years must elapse before the danger of dying from tuberculosis is past. Many of the deaths are from lung or general miliary tuberculosis. The radical surgeons claim that this high mortality is due to the tuberculous genitals which the conservative surgeons leave behind, yet they have presented no evidence that this mortality did not arise quite as much from the original process in the lung or other organs which induced the genital tuberculosis, as from the genital tuberculosis itself. Furthermore, few, if any, of their cases have been followed a sufficient length of time to show what the outcome will be. Certainly the results reported by Whiteside [8] are far from encouraging.

The foregoing discussion may not be entirely irrelevant to the subject of genital tuberculosis in children. It has been shown that these little patients are somewhat differently affected than are the adults, but after all their hematogenous and lymphatic channels are the same, they are equally susceptible if not more so than adults to infection, and yet the prostate and seminal vesicles still in a state of inactivity do not seem to be as frequently affected by tuberculosis as do the adult organs.

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8. Whiteside: Northwest Med. **18**:83-85, 1919.

TRAUMATIC DIAPHRAGMATIC HERNIA IN A GIRL OF EIGHT YEARS OF AGE*

MURRAY B. GORDON, M.D., AND DANIEL L. GOLANN, M.D.
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The extensive use of the roentgen-ray as an integral part of our diagnostic armamentarium has resulted among other things in an increase in the number of cases of diaphragmatic hernia reported in the literature of the past few years. This increase has not been due to a greater incidence of the condition, excluding those occurring during the late war, but to a more general use of the roentgen-ray together with improved technic of the roentgenologist. While it is not a common condition, still diaphragmatic hernia is probably not as rare as it was believed to be a few years ago. It may occur in two forms, congenital or traumatic. Congenital hernia is subdivided into true and false, the former being characterized by the presence of a hernial sac and the latter by its absence. The traumatic form is practically always without a sac. The size of the hernial opening may have a variable range from the smallest which permits the passageway of only one coil of small intestine to the larger rents of the diaphragm through which practically the entire gastro-intestinal tract may pass

The following case is an illustrative example of the pitfalls attending the proper diagnosis of the traumatic form.

REPORT OF CASE

History.—Josephine, 8 years old, gave a negative past history except for the injury because of which she entered the hospital. Five months previous to her coming to the Polhemus Clinic she was run down by an automobile truck, the wheels passing over her left groin. She was immediately removed to one of the best equipped hospitals in New York City. There was no evidences of any broken bones, shock or hemorrhage, either external or internal, immediately following the injury or at any other subsequent time. She remained in this first hospital for two weeks. While we have not been able to procure the records, it seems that a diagnosis was made of probable fluid in the chest as the mother remembers that the child was aspirated. No fluid was obtained. A roentgen-ray examination was made and the mother was informed that the child had a congenital dextrocardia.

The patient first appeared at our clinic, March 23, 1921, complaining of pain in the left shoulder, in the epigastrium and in the precordial region. Further questioning placed the time of the onset of the symptoms five months previous, in fact, immediately following the injury. To the injury itself not very much attention was paid as she seemed to have recovered from it without any apparent ill effects. From that time on, she was troubled with pain in the epigastrium appearing immediately after meals and lasting from one-half to several hours at a time. This pain gradually became less intense so that

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* From the Department of Pediatrics, Long Island College Hospital.

at the time of the examination, it was not so severe. She also complained of pain referred to the left shoulder in the region of the spine of the scapula and over the tip of the acromion process, this being present almost constantly. She was nauseated but did not vomit. Dyspnea was present, becoming aggravated by exercise. Her appetite was poor, bowels irregular. No history of any cough, hemoptysis or hematemesis. She had lost weight since her injury, going down from 62 to 43 pounds.

Physical Examination. — She was a poorly nourished Italian girl with a pasty pallid appearance, pinched expression, eyes simulating an exophthalmos. Her breathing was short and rapid. Examination of the chest made in the erect position gave the following findings: Normal breathing obtained on the right side throughout and on the left side as far down as the fifth interspace, and from then on diminished breathing. Percussion showed a dulness in the area of diminished breathing, normal resonance over the remainder of the chest. The heart was found to be displaced to the right side with the apex beat in the fifth right interspace, cardiac dulness extending from the second to the fifth interspace, the right border about 2 inches to the right of the midsternal line, the left border about 1 inch to the left of the sternum. There were no murmurs. The sounds were clearer to the right of the sternum than to the left. The liver was on the right side and the spleen on the left, neither viscus being palpable.

Diagnosis.—A diagnosis of pseudodextrocardia due to some unknown thoracic condition was made and the girl was referred to the roentgen-ray department for an examination of the chest.

Roentgen-Ray Report. — We were quite surprised to be informed by Dr. A. L. Loomis Bell, the roentgenologist to the hospital, that we were probably dealing with a case of diaphragmatic hernia as shown by the following findings: Marked displacement of the heart toward the right side, an absence of the left diaphragm outline, and the presence of peculiar gas shadows in the left chest, which, with the absence of gas shadows in the abdomen, very strongly suggest the presence of a diaphragmatic hernia (Fig. 1).

It was then decided to study the case from the viewpoint of a possible diaphragmatic hernia. It was fully a month before we were able to continue with the case as the child had removed to New York. On her return to the clinic a complete examination with the new possibilities in view, disclosed an entirely different picture.

Examination in the Erect Position.—Inspection: The anterior posterior diameter of the chest showed a tendency to the barrel shaped type, with an inclination toward stooping, probably due to the chronic dyspnea present since her injury. The measurements of the right side of the chest were slightly in excess of those on the left, and the expansion on the right was also above that on the left.

Palpation: Somewhat less fremitus on the left.

Percussion: Normal resonance on the right and normal resonance on the left side as far down as the fifth interspace where it changed to dulness.

Auscultation: Normal breath sounds, though somewhat harsh, throughout the right side. On the left, breath sounds were normal down to about the fifth space but absent below that.

Examination in the Supine Position.—Inspection and palpation revealed the same findings as in the erect posture.

Percussion: Normal resonance on the right side. On the left side, hyperresonance was obtained in the upper portion, this decreasing and changing definitely in about the fifth interspace to tympany to fuse with the tympanitic note of the abdomen.

Auscultation: Normal breath sounds on the right side. The left side presented normal sounds at the apex and as far down as the first two ribs both anteriorly and posteriorly. Below the second space, breath sounds were present in some areas and suppressed in others down to about the fifth rib while below this they were entirely absent. Occasional gurgling was heard in the lower portions of the left lung, but no râles.

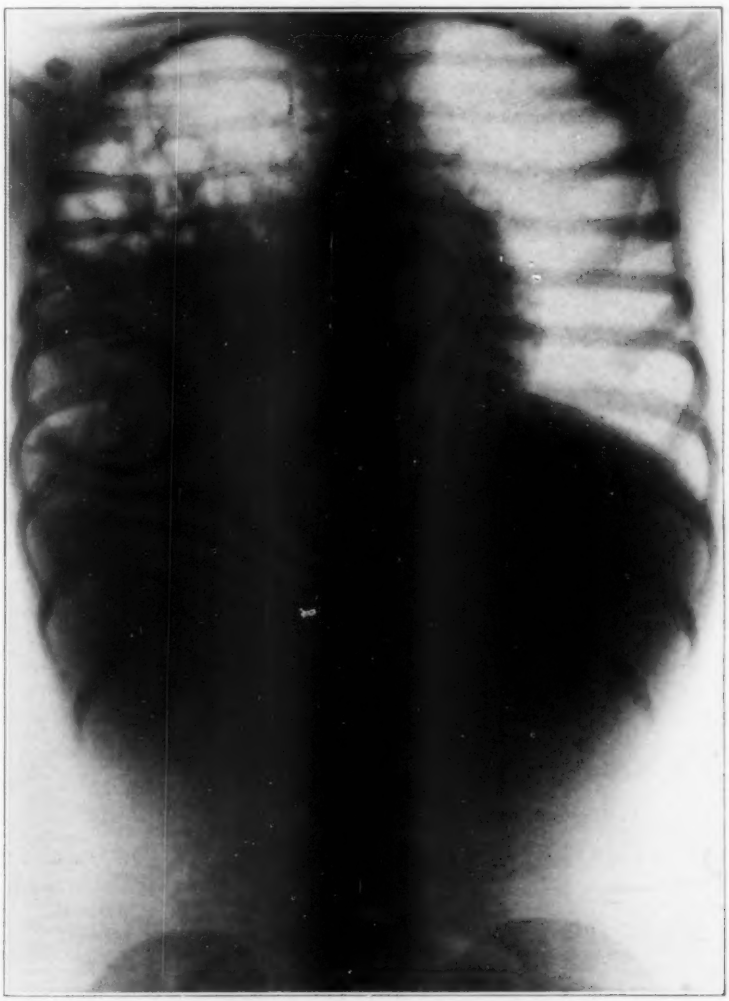

Fig. 1.—Diaphragmatic hernia is suggested.

Heart Examination.—Inspection: Faint apex beat visible in the fifth interspace on the right side. Percussion: Cardiac dulness extending from the second to the fifth space, the right border about 2 inches to the right of the midsternal line and the left border about 1 inch to the left of the sternum. Auscultation: Normal heart sounds heard with more intensity on the right than on the left of the sternum, the apex being found in the fifth right interspace within the nipple line.

Abdominal Examination.—In the erect position there was a fulness in the epigastrium while in the supine posture, the abdomen was scaphoid, somewhat tense and a falling in at the flanks. Percussion in the reclining position elicited tympany over the epigastrium and flatness over the remainder of the abdomen except over the liver where dulness was obtained extending 2 inches below the free border of the ribs. No masses were palpated.

Subsequent stereoscopic and roentgen-ray study by Dr. Bell demonstrated the following findings: Stereoscopic examination of the chest showed the presence of the stomach and part of the descending colon in the left chest. A later examination of the gastro-intestinal tract, including a small barium and buttermilk meal given by mouth and an opaque enema, showed the presence of the stomach in the normal abdominal position but the distal portion of the transverse colon and the proximal portion of the descending colon were entirely within the thorax on the left side (Fig. 2).

Blood.—Hemoglobin, 84 per cent. (Sahli); red blood cells, 4,432,000; white blood cells, 9,280, with 54.5 per cent. polymorphonuclears, 42 per cent. small lymphocytes, 1 per cent. eosinophils, and 2.5 per cent. transitionals.

Urinalysis.—Always negative.

Diagnosis and Treatment.—In the light of the roentgen-ray findings, coupled with the history of the injury in November and the subsequent clinical picture, we changed our diagnosis from pseudodextrocardia to traumatic diaphragmatic hernia and transferred the child to the surgical service of Dr. Emil Goetsch for operation. Dr. Goetsch operated on her, May 7, 1921. He will describe the operation and postoperative course in detail in a separate communication. He used the intrathoracic method, incising into the left seventh interspace. When the thorax was opened, it seemed as if the abdomen had been exposed to view, as the small intestines, large intestines, stomach and left lobe of the liver had all worked their way through a large rent in the diaphragm running anterioposteriorly on the left side. There were no adhesions.

She was discharged from the hospital, June 17, 1921, and appeared to have gained in weight and looked healthier than when she first came under observation. There was no dyspnea or cardiac embarrassment. Examination of her lungs showed a slight impairment of percussion note on the left side anteriorly with some slight amount of fluid still present in the chest. (This had developed subsequent to the surgical pneumothorax induced by the operation.) There was fairly good expansion of the left lung. The cardiac dulness extended 2½ inches to the left and about one-half inch to the right, with the apex in the fifth left interspace. The sounds were clear on the left side and in normal position.

DISCUSSION

Viewing our case from the present point of vantage, we can now readily observe where the mistakes in diagnosis were made and the reasons therefor. Presuming that the examiners had never seen a previous case of diaphragmatic hernia and were not acquainted with the clinical syndrome, still it is possible that a probable diagnosis, or perhaps even a correct one, could have been made had several points in the case been emphasized and properly interpreted.

History.—The history of an injury to the abdomen was clear cut and positive, and yet no one coupled the clinical picture with the injury. The reason for this may, perhaps, have been that she did not present any symptoms of internal injury such as shock or extensive hemorrhage.

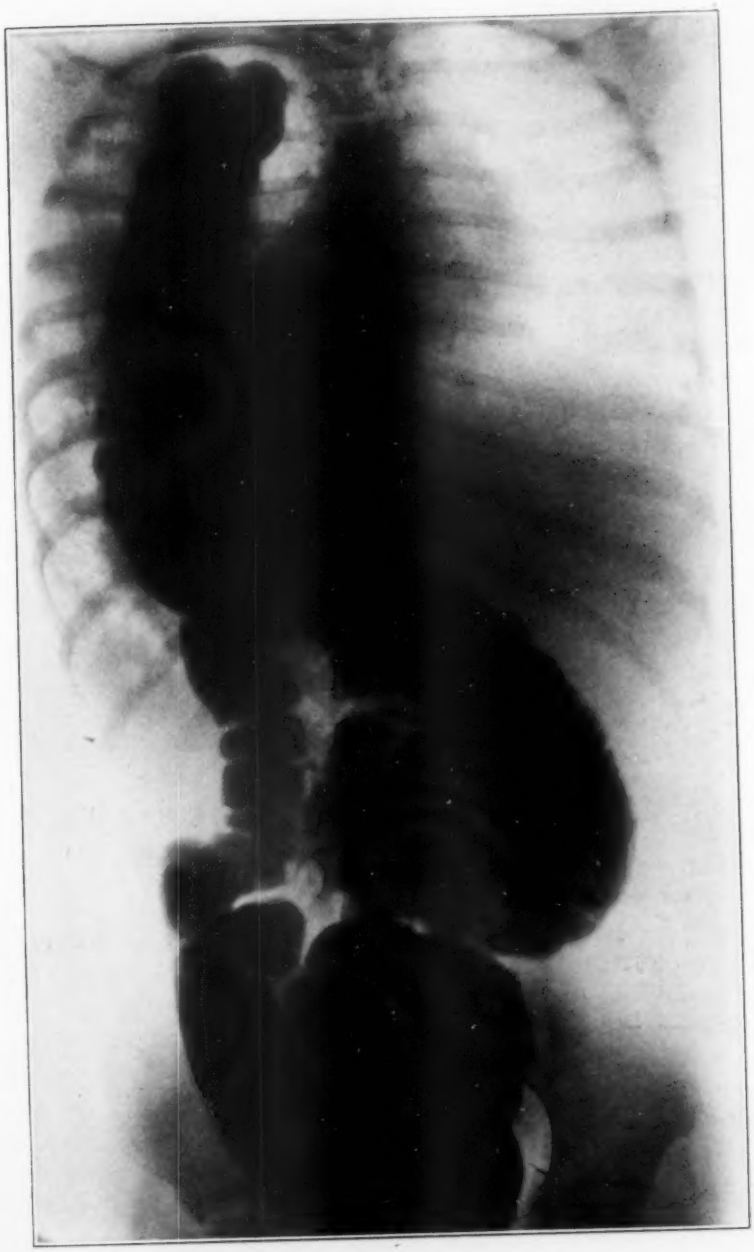

Fig. 2.—Contents of hernia.

Results of Injury.—The results of the injury were immediate and there is no reason to doubt that the rent in the diaphragm was not just as extensive at the time of her admission to the first hospital as at the time of the operation and presented the same symptoms and signs. The heart was displaced and the left lung was in partial collapse with an impairment of expansion. As there were no adhesions at any time, the left lung was free to expand to a greater or less degree, depending on the number of abdominal viscera present in the thorax, this, in turn, being governed by the position the child assumed. In the erect position, the abdominal contents dropped back into the abdomen, while in the supine position, they migrated to the thorax. This change of location of the abdominal contents gave rise to the difference in findings between the erect and supine positions. Aeration was present in the left apex and in occasional areas down to the fifth space, but below the fifth interspace there was an absence of breath sounds with dulness on percussion in the erect position and tympany in the supine. This dulness caused the first examiners to make a diagnosis of fluid and they accordingly aspirated but with negative results. Their conclusion is a common error in these cases. We ascribed this area of dulness not to fluid but to some unexplained chest condition which perhaps caused the dextrocardia.

Symptoms.—We found most of the symptoms generally present in diaphragmatic hernia such as dyspnea, displacement of the heart, rapid respiration, sunken abdomen and distention of the chest on the right side. While she did not show any cyanosis, her color was pasty and pallid.

Mistakes.—Errors in diagnosis, or rather, in interpretations of the signs and symptoms were due to: (1) Not enough importance was placed on the history of the abdominal trauma in relation to the subsequent symptom complex. (2) Examination of the chest was performed in the erect position and of the abdomen in the supine position. Had both positions been used in both regions, the difference in the signs would have suggested further search. (3) The acceptance of the diagnosis of dextrocardia, and the fact that the fluid was not found in the left side of the chest should have raised the question as to what caused dulness on that side in the erect and tympany in the supine posture. (4) Misinterpretation of the roentgen-ray findings. The first examiners evidently did not observe the partial collapse of the left lung and the absence of lung shadow below the fifth rib, in addition to the presence of gas shadows in this region.

CONCLUSIONS

The lessons to be learned from a study of this case are:

1. In every case of either true or false dextrocardia a roentgen-ray examination of the gastro intestinal tract with barium should be made.

2. Every case of dextrocardia should be examined completely in both the erect and supine positions.

3. A careful history should be obtained in every case of dextrocardia, especial emphasis being placed on the occurrence of any previous injury to the abdomen.

4. The possibility of traumatic diaphragmatic hernia should be considered in cases of severe abdominal injury when an increase of intra-abdominal pressure is present.

5. Because of the possibilities of strangulation, immediate operation is indicated in every case of traumatic diaphragmatic hernia, even in this case though six months had elapsed since the injury.

ISO-AGGLUTININS IN THE BLOOD OF THE NEW-BORN *

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In 1920 Minot and Weld,[1] working in this laboratory, examined for iso-agglutinins 160 specimens of blood from the umbilical cord obtained from the Boston Lying-In Hospital. They found that by testing both the serum and cells of these specimens against the cells and serum of all four groups that the groups could be established in 70 per cent. of these new-born infants. Their success in grouping this percentage of new-born infants seems to have due to a careful technic, and the recognition of weak agglutination. Because of the wide variation of their results from those reported by others, Minot suggested that I repeat this study and make certain further observations.

Most of the important advances in the knowledge of iso-agglutination have been made from studies of adult blood, and for this reason it is necessary to review some of these articles in this paper. The first important observations on iso-agglutination of human blood corpuscles were made independently by Shattock [2] and Landsteiner [3] in 1900.

In 1901, Landsteiner [4] reported further studies on iso-agglutination and showed that human beings could be divided into groups by the interreaction of their serum and red cells. His studies demonstrated only three of the four groups recognized today. These were termed A, B, and C. Landsteiner suggested at this time that iso-agglutinating properties occurred normally in human blood, and later he and Leiner [5] definitely showed that iso-agglutinins were not dependent on disease.

In 1902 von Decastello and Sturli [6] reported their studies on the blood of 155 persons. They believed that Landsteiner's classification was correct, but they noted that four persons failed to show iso-agglutinins in their serum and that the corpuscles of these persons were

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* From the Medical Services of the Massachusetts General Hospital.

* This paper is No. 21 of a series of studies on the physiology and pathology of the blood from the Harvard Medical School and allied hospitals, a part of the expense of which has been defrayed from a grant from the Proctor Fund of the Harvard Medical School, for the study of chronic disease.

1. Minot, G. R. and Weld, M.: Unpublished data in this laboratory.
2. Shattock, S. G.: J. Path. & Bact. **6**:303, 1900.
3. Landsteiner, K.: Zentralbl. f. Bakteriol. u. Parasitenk. **27**:361, 1900.
4. Landsteiner, K.: Wiener klin. Wchnschr. **46**:1132, 1901.
5. Landsteiner, K. and Leiner, K.: Zentralbl. f. Bakteriol. u. Parasitenk. **38**:548, 1905.
6. Decastello, A. and Sturli, A.: Münch. med. Wchnschr. **49**:1090, 1902.

agglutinated by all serums. These persons were considered as exceptions to the general rule, and no attempt was made to establish a fourth group.

A somewhat similar observation was made by Hektoen [7] in 1907. He reported that six persons out of fourteen belonging to Group III possessed no agglutinin in their serum. He further stated that not infrequently cells of Group III persons were agglutinated by serums of other Group III persons, and that this phenomenon did not occur in the other two groups.

The first observer to prove the presence of a fourth group was Jansky.[8] His studies were reported in 1907. In 1910, Moss [9] independently of Jansky also proved that there were four groups. The classification of Moss differs from that of Jansky in that Groups I and IV are reversed in the two classifications. Up to the present time there has been no record that any observer has compared the bloods of persons of Groups II and III as established by Jansky with the bloods of persons of Groups II and III as established by Moss. Hence, it is unknown whether Group II and III are identical or interchanged in the two classifications. The priority of establishing the four groups undoubtedly belongs to Jansky, and eventually Jansky's classification must be adopted. Because of the uncertainty of the identity of Jansky's Groups II and III, and particularly because the tests reported here were done with bloods whose group had been established with serum originally obtained from Moss, the classification of Moss is used in this paper. It may be stated as follows:

Group I. Corpuscles are agglutinated by serum of Groups II, III and IV. Serum agglutinates no corpuscles.

Group II. Corpuscles are agglutinated by serum of Groups III and IV. Serum agglutinates the corpuscles of Groups I and III.

Group III. Corpuscles are agglutinated by serum of Groups II and IV. Serum agglutinates corpuscles of Groups I and II.

Group IV. Corpuscles are agglutinated by no serum. Serum agglutinates corpuscles of Groups I, II and III.

Halban [10] was the first observer to study the relation of the iso-agglutinating properties of blood from new-born infants to those of mother's blood. He found that mother's serum not infrequently agglutinated infant corpuscles, whereas infant serum agglutinated

7. Hektoen, L.: J. Infect. Dis. **4**:297, 1907.
8. Jansky, J.: Klinicky Sbornik **8**:85, 1907; rev. in Jahresb. f. Neurol. u. Psychiat., 1907.
9. Moss, W. L.: Johns Hopkins Hosp. Bull. **21**:63, 1910.
10. Halban, J.: Wien. klin. Wchnschr. **13**:545, 1900.

mother's corpuscles rarely. He suggested that the iso-agglutinins did not originate in mother's blood but in the fetus.

Von Decastello and Sturli in 1902 also noted that the serum of new-born infants and infants under 6 months of age frequently failed to show agglutinins though the red cells were often agglutinated by adult serum.

Langer [11] in 1903, and Martin [12] in 1905, reported studies on the blood of mothers and infants. Their findings were quite similar to those reported by Halban.

In 1916, Cherry and Langrock [13] made a study of mother's blood and the blood of their new-born infants to determine if mothers would be suitable donors for their infants if transfusion were necessary. They mixed 0.5 c.c. of serum from the mother with 0.5 c.c. of a 5 per cent. suspension of infant cells which had been washed in physiologic sodium chlorid solution. Similar preparations were also made with infant serum and mother's cells. These mixtures were made in tubes and examined macroscopically. These investigators studied thirty-four cases in this way, and failed to observe either agglutination or hemolysis. They, therefore, concluded that mothers are safe donors for their infants.

L. and H. Hirschfeld,[14] in 1919, stated that the agglutinable properties of red cells appear early in embryonic life, e.g. a six months fetus. They believed iso-agglutinins did not appear until the second year.

In 1920, Happ [15] reported his studies on the appearance of iso-agglutinins in infants and children. He apparently mixed equal quantities of serum and 5 per cent. cell suspension and observed the reactions microscopically in hanging drop preparations. His cases were arranged in series according to age. In fifteen new-born infants the iso-agglutination group was determined only once from the characteristic behavior of both the serum and cells. Sixty-seven tests on forty-nine infants, including new-born infants and infants up to one month of age, showed only eight who could be grouped by both serum and cell reactions. One of these belonged to Group IV (Moss classification); the remainder belonged to Group I. Only seven infants showed iso-agglutinins in their serum, though the corpuscles of thirty-two were agglutinated by adult serum.

Happ stated that infant corpuscles may be agglutinated, by serum of Group IV only, or by serum of Groups II and IV or Groups III and IV, whereas the serum of the corresponding specimens may fail

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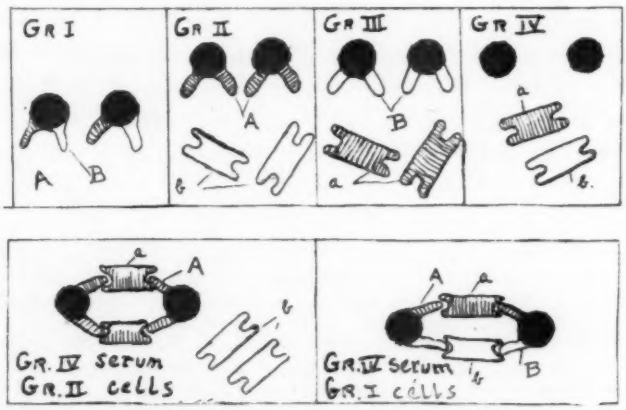
11. Langer, J.: Ztschr. f. Heilk. Abt. inn. Med. **24**:111, 1903; rev. by Happ.[15]
12. Martin, E.: Zentralbl. f. Bakteriol. u. Parasitenk. **39**:704, 1905.
13. Cherry, T. H. and Langrock, E. G.: J. A. M. A. **66**:626 (Feb. 26) 1916.
14. Hirschfeld, L. and H.: Lancet **1**:675, 1919.
15. Happ, W. M.: J. Exper. M. **31**:313, 1920.

to show iso-agglutinins. Happ was able to establish the group by both serum and cell reactions in 22.7 per cent. of infants between one and 3 months of age, in 31.8 per cent. of infants from 4 to 6 months and in 69.7 per cent of infants aged from 6 months to one year. After the first year the group could be established in practically every case,

Unger [16] has recently reported his studies on iso-agglutination. One hundred and nineteen of his cases were in children varying in age from one day to 4 years. He was able to demonstrate iso-agglutinins in the serum of only three infants out of twenty-four under one month of



Diagramatic representation of blood groups, and process of iso-agglutination. A and B are the receptors of red cells; a and b are the iso-agglutinins.

age, or 12.5 per cent. He stated that the percentage of children whose serum contained agglutinins increased with age, but reached the adult average only between the second and fourth years of life.

Attempts to explain the phenomenon of iso-agglutination have been made by numerous observers. Landsteiner, to whom the credit of initiating the study of iso-agglutination is due, advanced the theory that there were two iso-agglutinins and two receptors in human blood. These properties were regarded as more or less definite bodies in correspondence with Ehrlich's views on immunity. Since the appearance of Landsteiner's paper most of the workers on this subject have attempted either to prove or disprove Landsteiner's theory. The prevailing opinion seems to have been to consider Landsteiner's view correct, but some observers, for example, Moss and Hektoen, have considered that there are three iso-agglutinins rather than two. Studies on this subject have in large measure been based on the fact that the iso-agglutinating properties of serum can be removed by absorption

16. Unger, L. J.: J. A. M. A. **76**:9 (Jan. 1) 1921.

with proper cells. Studies reported by Koeckert,[17] Unger and others seem definitely to prove that there are two iso-agglutinins and two receptors in human blood. The studies reported on in this paper also give evidence to substantiate this view. The origin of these properties of human blood was much discussed by the earlier writers on this subject. At the present time the theory advanced by von Dungern and Hirschfeld[18] that these properties are inherited in accord with mendelian principles is generally accepted. The diagrams, the essentials of which are borrowed from the paper of L. and H. Hirschfeld, show in a diagrammatic manner the different blood groups and the process of iso-agglutination.

I have determined the frequency of the establishment of the iso-agglutination groups and noted certain other aspects of iso-agglutination in specimens of blood from 197 new-born infants. The technic employed did not differ radically from that used by Minot and Weld.

TECHNIC

The blood was obtained at the time of birth, under aseptic precautions, from the placental end of the cord. Specimens were tested twice a week, and were often from two to four days old when tested. So far as could be seen, these differences did not importantly affect the agglutinins in the serum nor the agglutinable properties of the red cells. The blood was centrifuged and the serum pipetted off. The clot was then shaken with 0.9 per cent. sodium chlorid solution and a suitable suspension of corpuscles made. In every case the serum was tested with adult cells of all four groups and the cells with serums of the four groups. Adult corpuscles were obtained for each day's work from a needle puncture of the ear lobe; the blood was first received in physiologic sodium chlorid solution to which had been added 1.5 per cent. sodium citrate. These cells were twice washed in 0.9 per cent. sodium chlorid solution and a 5 per cent. suspension was made. The known serums were not obtained fresh for each day's work, but were put up in sterile containers and kept cool until needed. This serum will be referred to as stock serum. It was frequently tested with known group cells to be sure that its activity was unimpaired.

The actual technic employed in performing the test for iso-agglutination seems to be important and may be the determining factor between the results reported here and the results reported by other observers. It may be well to recall here, for example, that Cherry and Langrock performed their tests in tubes and examined their preparations macroscopically; Happ, on the other hand, examined his hanging drop preparations microscopically but apparently used equal amounts of serum and cell suspension. In the present study ordinary glass slides and cover slips were used for the sake of convenience, rather than the hanging drop method, and from ten to twelve times as much serum

17. Koeckert, H. L.: J. Immunol. **5**:529, 1920.

18. Von Dungern, E. and Hirschfeld, L.: Ztschr. f. Immunitätsforsch. u. Exper. Therap. **6**:284, 1910.

as cell suspension was used in the individual tests. A comparison of the hanging drop and flat slide methods was made by testing the same mixtures of serum and cells. In twenty instances in which this comparison was made essentially similar results were obtained. Apparently, either the flat slide or hanging drop method is suitable for this kind of investigation but the macroscopic method is not. The infant cell suspension was mixed in the proportion of about one part to three with stock serum and covered with a cover slip. These preparations were examined microscopically in a few minutes to determine if agglutination had occurred. If the cells were definitely agglutinated, the result was recorded as positive. If agglutination had not occurred, the cover slip was lifted, the preparation agitated and in a minute or so examined again. This agitation was done because it seemed to facilitate agglutination. As a rule, agglutination will occur in a minute or so if serum with a high agglutinating titre is used. If a less active serum is used, up to twenty minutes, rarely longer, may be required before agglutination is visible. This weak or slow appearing agglutination is sometimes accelerated by incubating the preparation at 37 C. The results with infant cells and stock serum were always definite and no difference in agglutinability could be detected between infant cells and the cells of adults. The evidence brought out in this study, as pointed out later, strongly suggests that the cells of new-born infants have their full quota of receptors.

In testing infant serum care must be employed because, as a general rule, infant serum is much weaker in its agglutinin content than adult serum. The technic followed here in testing infant serum was planned to facilitate the recognition of these weak agglutinins. A large drop of infant serum was dropped on the slide, and a small drop of the 5 per cent. known cell suspension, equal in quantity to from one tenth to one twelfth of the serum, was placed beside it. A cover slip was placed over the two drops and tilted from side to side to bring as much of the serum into contact with the cell mass as possible without scattering the cells any more than necessary. This procedure is an attempt to allow the cells to absorb the agglutinins present and yet remain close enough together to be agglutinated. The preparations were allowed to stand for about ten minutes and then were examined under the low-power lens of the microscope for the presence or absence of agglutination. Before agglutination occurred it was often necessary to agitate the preparation by lifting the cover slip. This not infrequently was repeated several times at intervals of a few minutes and positive results sometimes did not occur for thirty minutes. No questionable agglutinable reactions were recorded as positive unless the control preparations of infant serum and Group IV cells were definitely nega-

tive. The agglutinating titre of infant serum is usually much lower than that of adult serum as is evidenced by the necessity of using larger amounts of serum and the frequency with which incomplete agglutination is observed.

All of the 197 infant blood specimens could be placed into four groups in accord with the classification of Moss, if the agglutinating action of stock serum on infant cells is accepted as satisfactory criteria for group determination. It was not possible to confirm this grouping in all specimens by noting the agglutinating action of infant serum on known group cells; 78.7 per cent. of the specimens, however, could be so confirmed. Because the group corresponded in 78.7 per cent., and because reactions of infant cells with stock serum were always definite, it is believed that the agglutinative response of infant cells to known group serums is sufficient evidence to permit classification. The correctness of this assumption was further borne out by the percentage of occurrence of the different groups. The classification of the 197 specimens of infant blood is shown in Table 1:

TABLE 1.—CLASSIFICATION OF INFANT BLOODS

Group I	3.5%
Group II	35.5%
Group III	13.7%
Group IV	47.2%

These figures are quite similar to those Minot and Weld found last year.

Culpepper and Ableson[19] have recently reported the typing of 5,000 persons according to the classification of Moss. The occurrence of the different groups in their series, closely corresponds with the figures for adults reported by others in this country. Their figures are shown in Table 2:

TABLE 2.—CLASSIFICATION OF ADULT BLOODS (CULPEPPER AND ABLESON)

Group I	5.18%
Group II	36.06%
Group III	14.28%
Group IV	44.48%

A comparison of these two tables shows that the different groups occurred with approximately the same frequency in the new-born as they do in the adult.

19. Culpepper, W. L. and Ableson, M.: J. Lab. & Clin. M. **6**:276, 1921.

Certain significant findings were made in this study concerning the nature and time of appearance of iso-agglutinins in human blood. These findings are best brought out by a discussion of the agglutinating activity of infant serums of the different groups.

GROUP I: The cells of this group were agglutinated by all serums except, of course, the serum of their own group which contains no iso-agglutinins. In this study none of the five bloods whose cells were agglutinated by serums of Groups II, III and IV had any iso-agglutinins in their serum, and therefore the group was established in 100 per cent.

GROUP II: Bloods whose cells were agglutinated by serums of Groups III and IV, were placed in this group. The serums of fifty-one of the seventy bloods placed in this group (72.8 per cent.) agglutinated cells of both Group I and Group III. Of the nineteen serums which did not agglutinate Group I and Group III cells, only three showed any agglutinating power; one agglutinated Group I cells and two agglutinated very slightly Group III cells. It would seem that iso-agglutinin "b," which occurs alone in this group, is equally effective against Group I and Group III cells.

GROUP III: Bloods whose cells were agglutinated by serums of Groups II and IV were placed in this group. The serum of twenty-one of the twenty-seven bloods placed in this group (81.5 per cent.), agglutinated cells of both Group I and Group II. Of the six serums which did not agglutinate Group I and Group II cells, two agglutinated Group II cells but none agglutinated Group I cells alone. Six of the serums which agglutinated both Group I and Group II cells showed a higher agglutination titre for Group II cells than for Group I cells. These findings would suggest a preponderance of receptor "B" in the Group I cells used in this study, or an unexplained peculiarity of iso-agglutinin "a."

GROUP IV: The cells of bloods placed in this group were not agglutinated by any serum. The serums of seventy-six of the ninety-three bloods placed in this group (81.7 per cent.), agglutinated cells of Groups I, II and III. All of the serums (100 per cent.) placed in this group showed some agglutinating activity. An analysis of the iso-agglutinating activity of these serums on cells of Groups I, II and III gives strong confirmatory evidence to the theory that there are two iso-agglutinins in Group IV serum and that they are similar in nature to the iso-agglutinins occurring in the serums of Groups II and III. Table 3 shows in detail the iso-agglutinating activity of serums placed in Group IV which did not agglutinate equally cells of all three groups:

TABLE 3.—Iso-Agglutinating Activity of Group IV Serums Which Did Not Agglutinate Equally Cells of All Three Groups

Serum agglutinating cells of Group I only	0
Serum agglutinating cells of Group II only	3
Serum agglutinating cells of Group III only	1
Serum agglutinating cells of Groups I and II	2
Serum agglutinating cells of Groups I and III	10
Serum agglutinating more markedly cells of Groups I and II than cells of Group III	0
Serum agglutinating more markedly cells of Groups I and III than cells of Group II	13
Serum agglutinating more markedly cells of Group II than cells of Groups I and III	8

An analysis of the results recorded in this table shows that Group IV serum at birth may have either iso-agglutinin "a," iso-agglutinin "b," or both; and that if both occur they may have essentially the same strength of agglutinating power or one may be more potent than the other.

In reference to the time of appearance of iso-agglutinins of human blood, it may be noted that at least six of the specimens were from premature infants. Most of these infants were estimated to be from two to three weeks premature and one was a seven months' fetus. Of the bloods of the six premature infants, five, including that of the seven months' fetus, could be grouped completely, and the sixth showed some agglutinating activity of the serum. The results recorded above show that a great many more new-born infants have iso-agglutinins in their serums than has previously been demonstrated.

The chief sources of possible error encountered in any test for iso-agglutination are: (1) the appearance of the rouleaux phenomenon; (2) the clumping due to drying at the edges of the cover slip; (3) auto-agglutination; (4) the occurrence of iso-hemolysis; and (5) a rather indefinite type of agglutination described as occurring in mixtures of serum and cells of the same group.

The rouleaux phenomenon is ordinarily not confusing. In this study when much rouleaux formation was present and agglutination was questionable, the reaction was considered negative. The appearance of false clumping due to drying as a rule causes very little difficulty because if the tests are set up properly they can usually be read as positive or negative before the preparation dries to any significant extent. Strong auto-agglutination was not observed, but a weak form of auto-agglutination was observed in 14.2 per cent. of the specimens of infant blood. The interreaction of serum and cells of the same groups was not observed as a separate phenomenon from weak auto-agglutination.

Strong auto-agglutination has been recognized for some time. One of the best papers on the subject appeared in 1918 by Clough and Richter.[20] It was shown by these observers that this peculiar agglutinin was dependent for its activity on a lowered temperature. When the temperature was lowered, the serum containing this agglutinin clumped not only the red cells of any person but also clumped animal cells, e. g., rabbit, guinea-pig and sheep. When the temperature was raised to approximately body temperature the ability to hold the cells in clumps was lost. Infant serum showing the type of agglutination referred to as weak auto-agglutination would clump cells of all four groups and for this reason could easily be recognized. Frequently this form of agglutination would not occur until the preparation had been agitated by lifting the cover slip, though often very slight movement of the preparation was sufficient to cause the cells to be clumped actively. In the earlier instances of this phenomenon the preparations were allowed to stand a few minutes and then were reexamined; the cells clumped by iso-agglutinins would remain together while the "auto" agglutinated cells would usually come apart but would be lying close together in a pattern arrangement such as may occur with serum with weak iso-agglutinating power. Very slight agitation of the preparation would, however, cause these cells to come together again. On several occasions when this type of agglutination was observed, the serums were tested against their own cells. It was observed that these cells were agglutinated in every instance. It was this observation that suggested the true nature of the phenomenon. Acting on this suggestion, preparations showing this type of agglutination were gently warmed by holding the slide between the fingers above a small flame. This operation quickly separated the "auto" agglutinated cells but did not appear to affect the cells clumped by iso-agglutinins. As a further study of this phenomenon, preparations were chilled by placing them in the ice-box. This procedure seemed definitely to increase the activity of the auto-agglutinin and further confirmed the impression that the phenomenon was in reality a weak form of auto-agglutination.

Another phenomenon causing possible error in a test for iso-agglutination is the occurrence of iso-hemolysis on the glass slides at room temperature (from 20 to 22 C.). Twenty-seven of the 197 serums examined showed this phenomenon. In some instances hemolysis occurred so rapidly the twice washed cells were not seen agglutinated. In other instances, when agglutination had occurred quickly, one was able to watch the disintegration of the clumps and the dissolution of the cells under the action of the hemolysin. Several preparations had

20. Clough, M. C. and Richter, I. M.: Johns Hopkins Hosp. Bull. **79**:86, 1918.

to be placed in the cold in order to decrease the action of the hemolysin and thus permit the visible action of the agglutinin. In one instance it was necessary to destroy the complement by heating the serum before the weak agglutinin could manifest its presence. This same phenomenon has been observed occasionally with serum and unwashed cells under the same conditions, but usually the small amount of plasma with such cells furnishes enough anti-hemolysin to prevent it.

The practical application of this study will be found in its relation to the problem of transfusion in young infants. Transfusion is now recognized as a curative measure in hemorrhagic disease of the newborn and is regarded as a valuable procedure in the treatment of severe anemias. Marriott [21] recently recommended transfusion as a valuable measure in the treatment of "athrepsia" because of the diminished protein concentration of the plasma and the lowered blood volume found in this condition. Transfusion of an incompatible blood into an adult may be disastrous. The blood of prospective donors is now universally tested for compatibility with the blood of the patient before transfusion is done. Because of the prevailing misconception that iso-agglutinins and iso-hemolysins are not present in the blood of new-born infants, it is often conceded that it is not necessary to make compatibility tests before transfusing infants. It is undoubtedly true that the danger of transfusing unsuitable blood into an infant is much less than it is in the case of adults. This has been demonstrated by clinical experience and by the laboratory evidence that iso-agglutinins are, as a general rule, much weaker in infant serum than they are in adult serum. The findings of this study, and those of a simultaneous study on iso-hemolysins in the following paper,[22] would, however, suggest that there is an element of danger in transfusing infants from untested donors. Iso-agglutinins occasionally occur in relatively high concentration in blood of new-born infants and iso-hemolysins, when they are present, are frequently as strong as those seen in adult blood. It is therefore desirable that iso-agglutination tests should be done before transfusion into infants, and the person selected as donor should belong either to Group IV or to the same group to which the infant's cells belong. If the group serum for doing these tests is not available, carefully conducted tests with infant's serum and donor's corpuscles represent the minimum requirement. As an additional control, infant's cells and donor's serum may also be tested for compatibility. Agglutination should be looked for with the microscope and not macroscopically. The person whose cells are not agglutinated by the infant's serum may presumably be used without danger of serious reaction,

21. Marriott, W. McK.: Am. J. Dis. Child. **20**:461 (Nov.) 1920.
22. Jones, B. B.: Am. J. Dis. Child. **22**:598 (Dec.) 1921.

though the person who belongs to the same group as the recipient is, when there is a choice, the best donor.

SUMMARY AND CONCLUSIONS

1. Seventy-eight and seven-tenths per cent. of the 197 specimens of infant blood examined could definitely be placed in one of the four recognized iso-agglutination groups since this number gave the complete group reactions when the infant cells were tested with serum of the four groups and the infant serums were tested with known group cells. This percentage of new-born infants having their group established is much higher than has previously been reported. The results seem to be dependent on a technic which permits the recognition of weak agglutinins.

2. Evidence is given which strongly suggests that the cells of new-born infants have their full quota of receptors. If this is correct then 100 per cent. of new-born infants can be grouped satisfactorily by testing their cells with known group serums.

3. Infant serum of specimens whose cells fell into Group II definitely agglutinated cells of Groups I and III in 72.8 per cent. of the cases examined. The serum of bloods similarly placed in Group III definitely agglutinated cells of Groups I and II in 81.5 per cent. The serum of bloods placed in Group IV agglutinated cells of Groups I, II and III in 81.7 per cent.

4. A study of the iso-agglutinins of Group IV infant serums shows that either iso-agglutinin "a," iso-agglutinin "b," or both may be present. If both are present, they may occur in the same strength or one may be stronger than the other.

5. Iso-agglutinins have been demonstrated in the blood of a seven months' fetus.

6. A weak form of auto-agglutination was observed in 14.2 per cent. of the infant serums examined.

7. Thirteen and seven-tenths per cent. of the infant serums hemolyzed washed cells in cover glass preparations at room temperature.

8. The demonstration of strong iso-agglutinins and iso-hemolysins in the serum of new-born infants indicates the advisability of making proper tests for compatibility before selecting a donor for transfusion in infants.

I wish to express my thanks to the staff of the Boston Lying-In Hospital, particularly the House staff, for the samples of blood used in this study; and to Drs. George R. Minot, Roger I. Lee, and William L. Moss for advice and assistance.

ISOHEMOLYSINS IN HUMAN BLOOD, WITH ESPECIAL REFERENCE TO THE BLOOD OF THE NEW-BORN *

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The isohemolytic properties of human blood have been studied from time to time by various observers. Relatively few papers have appeared on this subject, however, in comparison to the large number on the closely related subject iso-agglutination. There is a marked difference in the results reported by different observers on the percentage of occurrence of isohemolysins in the blood of adults. The question of isohemolysins in the blood of new-born infants does not seem to have been investigated adequately. This study was begun to determine if isohemolysins had developed in the blood of new-born infants. It was subsequently extended to include a series of adults for the purpose of comparison under the same conditions of technic.

The first recorded observation on isohemolysis was made by Maragliano [1] in 1892. He stated that the serums of persons suffering from essential anemia, carcinoma, malaria, etc., have the property of destroying normal erythrocytes and changing hemoglobin to hemotoidin.

Following Maragliano's work, no noteworthy studies on isohemolysis were made until after Shattuck's [2] and Landsteiner's [3] observations on iso-agglutinins in 1900. Their work stimulated many investigators to study iso-agglutination and also reawakened the interest of the scientific world for the hemolytic properties of human serum. Isohemolysins were at first considered as dependent on disease, but this theory was largely discarded after Landsteiner and Leiner [4] in 1905 showed that approximately 50 per cent. of both normal and sick children had isohemolysins in their serum. They employed for their study the red cells of three persons, who from their description could fall into Groups II, III and IV of Moss' [5] classification. These cells were washed in

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* From the Medical Services of the Massachusetts General Hospital.

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1. Maragliano: Verhandlungen des XI Kongr. f. Inn. Med., 1892.
2. Shattuck, S. G.: J. Path. & Bacteriol. **6**:303, 1900.
3. Landsteiner, K.: Zentralbl. f. Bakteriol. u. Parasitenk. **27**:361, 1900.
4. Landsteiner, K. and Leiner, K.: Zentralbl. f. Bakteriol. u. Parasitenk. **38**:545, 1905.
5. Moss, W. L.: Johns Hopkins Hosp. Bull. **21**:63, 1910.

physiologic sodium chlorid solution and a 2.5 per cent. suspension was made. It was observed that iso-hemolysis was not found as often as iso-agglutination and that a given serum might hemolyze either one or two of the three types of cells used. They stated that strong iso-hemolysis was seen only when agglutination had occurred.

It has become well recognized that human beings can be divided into four groups according to the way their serums agglutinate the corpuscles of others and by the agglutinability of their corpuscles with different serums. Moss' classification was followed in this study and is stated in Table 1:

TABLE 1.—Moss' Classification

Cells	Serum			
	I	II	III	IV
I	—	+	+	+
II	—	—	+	+
III	—	+	—	+
IV	—	—	—	—

+ = agglutination, — = no agglutination.

In addition to his studies on iso-agglutination Moss also reported observations on isohemolysis. He used a technic quite similar to that employed by Landsteiner and Leiner, except that he used a 5 per cent. instead of a 2.5 per cent. suspension of corpuscles. He observed isohemolysins in 25 per cent. of 100 serums examined and stated that isohemolysis never occurred independently of iso-agglutination. Moss expressed the belief that iso-agglutinins and isohemolysins had a common origin. He showed that all serums contained an antihemolysin that protected the homologous corpuscles and those of the same group from dissolution by any other serum. Moss believed that antihemolysin was an anti-amboceptor which acted by uniting with the cytophylic group of the hemolysin, thus blocking it off from the red cell, and that the anti-hemolysin consisted of receptor groups from the red cells, which had been cast off from the cell or set free by the physiologic or pathologic destruction of the corpuscles.

Von Dungern and Hirschfeld,[6] working at the same time as Moss (1910), studied the iso-agglutinins and their receptors in human blood. They confirmed the theory originally advanced by Landsteiner[7] that only two iso-agglutinins, "a" and "b," and two receptors, "A" and "B," were present in human blood. They showed that the following combinations might occur in the blood of any one individual: A and B, A and b, B and a, and a and b, and suggested that these properties are inherited in accord with mendelian principles.

6. Von Dungern, E. and Hirschfeld, L.: Ztschr. f. Immunitätsforsch. u. Exper. Therap. **6**:284, 1910.

7. Landsteiner, K.: Wien. klin. Wchnschr. **46**:1132, 1901.

Grafe and Graham [8] showed that the isohemolysins corresponded to the "a" and "b" iso-agglutinins, and that disease had no etiologic relation to their presence in the serum.

Cherry and Langrock [9] studied the blood of mothers and their newborn infants. They mixed equal amounts of infant's serum and a 5 per cent. suspension of washed corpuscles from the mother in one tube, and similar amounts of infant's cells and mother's serum in another. In thirty-four such tests they failed to observe either iso-agglutination or isohemolysis. Their findings are not in accord with those of other observers.

Recently, Williams [10] emphasized the fact that isohemolysis never occurs without associated iso-agglutination. He stated that when a serum has strong agglutinative power it may cause slight or no noticeable hemolysis in the quantity used. "The hemolysin is present, however, and can always be demonstrated when sufficient complement is present by increasing the amount of serum."

It is generally conceded that there are two isohemolysins among humans just as there are two isoagglutinins. Isohemolysins resemble the acquired hemolysins that originate after the injection of foreign blood into the body of an animal, in that they are thermostabile, specific, and that they require complement for their action. Complement is a nonspecific thermolabile substance found in all fresh serums. The process of isohemolysis consists of the attachment of the isohemolysin-complement complex to the corresponding receptor of a suitable red cell and the subsequent dissolution of the cell.

In this study the following technic was followed in studying the isohemolysins of both adult and infant blood: 0.2 c.c. of serum was added to 0.1 c.c. of a 5 per cent. suspension of known group cells which were twice washed and suspended in 0.9 per cent. sodium chlorid solution. Each serum was tested against cells of all four groups. The serums examined were assumed, because of their freshness, to have enough complement to insure hemolysis but in some instances this may not have been the case. (If this factor had been strictly controlled the results with adult serums might have more closely paralleled those of Williams.) The tubes containing the serum and cells were placed in a water bath at 37 C. for two hours and then were examined for the presence or absence of isohemolysis. Unless the cells had completely settled in this time the preparation was gently shaken and centrifuged slowly. This procedure permitted slight degrees of hemolysis to be detected which might otherwise have passed unnoticed, and in no instance did it seem to cause traumatic hemolysis.

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8. Grafe, E. and Graham, D. A. L.: Münch. med. Wchnschr. **58**:2257, 1911.
9. Cherry, T. H. and Langrock, E. G.: J. A. M. A. **66**:626 (Feb. 26) 1916.
10. Williams, W. C.: J. Exper. M. **32**:159, 1920.

There were 265 specimens in all examined. Of this number 144 were from adults, and 121 were from new-born infants. Of the 140 samples of adult blood belonging to Groups II, III and IV, 124, or 88.5 per cent. contained demonstrable isohemolysins. Of the twenty specimens (13.9 per cent.) which did not show isohemolysins, four belonged to Group I, which should have no isohemolysins, twelve to Group II, and two each to Groups III and IV. Of the 121 infant specimens, 117 belonged to Groups II, III and IV, and thirty-two of these showed isohemolysins, or 27.3 per cent. Table 2 shows the isohemolytic reactions of twelve adult and twelve infant serums which were chosen as representatives of the two kinds of blood.

TABLE 2.—Isohemolytic Reactions of Twelve Representative Adult Serums and Twelve Representative Infant Serums

	Adult Serum*					Infant Serum*			
Group	I	II	III	IV	Group	I	II	III	IV
IV	++	++	++	—	IV	+	+++	—	—
III	tr	++	—	—	IV	tr	++	—	—
IV	+	++	+	—	IV	tr	tr	tr	—
IV	+	tr	+	—	III	+	+++	—	—
III	tr	++	—	—	IV	—	tr	—	—
IV	++	++	++	—	IV	tr	tr	tr	—
II	+	—	+	—	IV	tr	—	tr	—
II	tr	—	tr	—	IV	+	++++	tr	—
II	+++	—	+++	—	II	++	—	++	—
IV	++	+	++	—	III	—	+	—	—
IV	+++	+++	+++	—	IV	—	tr	—	—
II	+	—	+	—	III	?tr	++	—	—

* The groups to which the serums belonged are indicated in the left hand column. The degrees of hemolysis of known group cells by the different serums are indicated in the other four columns.

An examination of this table brings out the chief differences between the isohemolytic reactions of adult and infant serums. It will be observed that adult serum, as a general rule, shows slightly stronger hemolytic power than infant serum. However, infant serum may contain isohemolysins which are quite as strong as those seen in adult serum. Adult serums of Group IV more frequently have both isohemolysins "a" and "b" than do infant serums of this same group, but the same tendency for one isohemolysin to predominate is seen in both. Serums of Groups II and III, irrespective of the age of the person from whom they are obtained, show the same types of hemolytic reactions. The chief difference between adult and infant serums of these groups lies in the frequency of occurrence of isohemolysins. An analysis was made of the results obtained in the study of infant serums.

Four serums belonged to Group I, which never has isohemolysins, so, naturally, the tests were negative in all. Forty serums belonged to Group II. Of these forty specimens only two, or 5 per cent., showed the presence of isohemolysin. Isohemolysin "b," which occurs in this group, seems to be equally effective against cells of both Group

I and Group III. (This fact was brought out more effectively from the study of adult serums.) Twenty serums belonged to Group III. Of these twenty serums there were seven, or 35 per cent., which contained isohemolysin. The isohemolysin "a," which occurs in this group, was much more effective against the Group II cells than against the Group I cells used. In three instances only Group II cells were hemolyzed, while in the other four instances the lytic action on Group II cells was much more marked than on Group I cells. This finding would suggest a preponderance of receptor "B" in the Group I cells used.

Fifty-seven serums belonged to Group IV. Of these fifty-seven specimens, twenty-three, or 40.3 per cent., showed isohemolysins. The results of a study of this group are shown in Table 3.

TABLE 3.—ANALYSIS OF ISOHEMOLYTIC REACTIONS OF TWENTY-THREE INFANT SERUMS OF GROUP IV WHICH CONTAINED ISOHEMOLYSINS

Group IV serum causing essentially same degree of hemolysis of Groups I, II and III cells (a = b)	5
Group IV serum causing hemolysis of Group II cells only (a)	5
Group IV serum causing greater hemolysis of Group I than Groups II and III (a = b)	1
Group IV serum causing greater hemolysis of Groups I and II than Group III (a > b)	1
Group IV serum causing greater hemolysis of Groups I and II than Group II (a < b)	2
Group IV serum causing greater hemolysis of Group II than Groups I and III (a > b)	4
Group IV serum causing hemolysis of Groups I and II only (a)	4
Group IV serum causing hemolysis of Groups I and III only (b)	1

An examination of these results shows that both isohemolysins can be demonstrated in the blood of the new-born. The isohemolysin "a," which occurs in Groups III and IV, is much more often present at birth than isohemolysin "b," which occurs in serum of Groups II and IV.

Because of the striking predominance of isohemolysin "a" at birth, the results in the adult series were examined to see if this same tendency had persisted in adult life. It has been shown that sixteen of the 140 adult specimens belonging to Groups II, III and IV failed to show demonstrable isohemolysins. Twelve of the sixteen specimens belonged to Group II, and two each to Groups III and IV. These figures show that the same tendency for isohemolysin "a" to predominate exists in adult blood, but to a much less degree, than in infant blood. An analysis of the isohemolytic reactions of fifty-five adult serums of Group IV, shown in Table 4, also demonstrates this modified tendency for isohemolysin "a" to predominate.

It has been suggested by Moss that iso-agglutinins and isohemolysins have a common origin. It is certainly true that these properties of serum are closely related and that isohemolysins never occur independently of iso-agglutinins. The development of isohemolysins, however, does not seem to keep pace with the development of iso-agglutinins, and the developmental difference between isohemolysins "a"

and "b" is in sharp contrast with that of iso-agglutinins "a" and "b." A study of iso-agglutinins in the serum of the new-born was reported in the previous paper.[11] It was shown in this study that iso-agglutinin "a" occurred about as frequently as iso-agglutinin "b," which is in contrast to the development of isohemolysin "a" over isohemolysin "b." Iso-agglutinins were demonstrated nearly three times as often as were isohemolysins in the same infant serum while in adult serum isohemolysins were demonstrated nearly as frequently as iso-agglutinins.

TABLE 4.—ANALYSIS OF ISOHEMOLYTIC REACTIONS OF FIFTY-FIVE ADULT SERUMS OF GROUP IV

Group IV adult serum hemolyzing equally Group II and III cells (a = b)	25.4%
Group IV adult serum hemolyzing Group II cells more markedly than Group III (a>b)	41.8%
Group IV adult serum hemolyzing Group III cells more markedly than Group II (a<b)	21.8%
Group IV adult serum hemolyzing Group II cells only (a)	9.9%
Group IV adult serum hemolyzing Group III cells only (b)	1.8%

The effect on the degree of isohemolysis by increasing the number of cells in the individual tests was also investigated. This study was made because a comparison of the technics of the various observers suggested that varying the number of cells in the tests might exert an influence on the detection of weak isohemolysins. As indicated above, the results of different observers on the percentage occurrence of isohemolysins in human blood are quite different. A fixed amount of adult serum was tested against varying amounts of the cells of another group. The first observations strongly suggested that an increased number of cells did tend to prevent hemolysis. The cells in this series were washed twice in physiologic sodium chlorid solution, and two suspensions, one of 5 per cent. and one of 20 per cent., were made. Parallel tests were made using 0.2 c.c. of each cell suspension and 0.2 c.c. of serum. Table 5 shows the groups of the serums tested and the results of the various tests:

TABLE 5.—EFFECT ON DEGREE OF ISOHEMOLYSIS BY INCREASING NUMBER OF CELLS IN TESTS

	5% Cells			20% Cells				5% Cells			20% Cells		
Group	II	III	IV	II	III	IV	Group	II	III	IV	II	III	IV
IV	+	—	—	?tr	—	—	II	—	+++	—	—	tr	—
II	—	++	—	—	tr	—	IV	tr	tr	—	—	—	—
II	—	tr	—	—	—	—	IV	++	tr	—	tr	?tr	—
IV	tr	++	—	—	—	—	II	—	tr	—	—	—	—
IV	tr	?tr	—	—	—	—	IV	+++	++	—	tr	?tr	—
IV	++	+	—	?tr	?tr	—	II	—	+++	—	—	tr	—
II	—	?tr	—	—	—	—	IV	tr	?tr	—	—	—	—
I	—	—	—	—	—	—	III	tr	—	—	—	—	—
IV	—	tr	—	—	?tr	—	II	—	+++	—	—	+	—
II	—	—	—	—	—	—	IV	+	+++	—	—	+	—
II	—	—	—	—	—	—	IV	++	+	—	tr	?tr	—
IV	+	?tr	—	?tr	—	—	II	—	++	—	—	tr	—

Degrees of hemolysis are as indicated in Table 2.

11. Jones, B. B.: Am. J. Dis. Child. **22**:586 (Dec.) 1921.

The next series of observations were made to determine if the greater number of cells carried enough antihemolysin to decrease the amount of hemolysis observed in tests with fewer cells. The cells were washed three times instead of twice as in the previous experiment, and 2.5 per cent. and 25 per cent. suspensions were made instead of 5 and 20 per cent. Despite the greater differences in number of cells in the parallel tests, the differences in degree of hemolysis were not so marked as in the first series. A final series of tests were made with the same serums and cells as were used in the second series but this time the cells were washed five times instead of three. The tests still showed a very slightly greater amount of hemolysis when fewer cells were used, but the differences were less than in the tests with thrice washed cells. It would seem that these results could be explained on the basis of incomplete removal of antihemolysin, and the tests demonstrate that it is not an easy matter to remove all antihemolysin. It seems quite possible in the light of these results that the varying reports in the literature on the frequency of isohemolysis are in part dependent on varying amounts of antihemolysin present in the tests of various observers.

One practical application of this study lies in its relation to the question of transfusion of young infants. This matter was rather fully discussed in the paper on iso-agglutinins. It was suggested in this paper that tests for compatibility should be done before selecting donors for transfusion in infants. The results of this study show that about one third as many new-born infants have demonstrable isohemolysins in their blood as do adults, and further stress the desirability of doing compatibility tests before transfusing infants. The chief danger of transfusing infants from untested donors lies in the fact that isohemolysin "a" is so frequently present at birth. Infants of Groups III and IV whose serums contain this hemolysin would presumably have hemolytic reactions if transfused from Group II donors.

SUMMARY AND CONCLUSIONS

1. One hundred and twenty-one specimens of blood from new-born infants and 144 specimens of adult blood have been examined for the presence of isohemolysins. Isohemolysins have been found in 27.3 per cent. of infant specimens of Groups II, III and IV, and in 88.5 per cent. of adult specimens of the same groups.

2. Besides the difference in frequency of occurrence of isohemolysins in adult and infant serums, the following differences were noted: (a) the isohemolysins of adult serums, as a general rule, appear to be slightly stronger than those seen in infant serums, but this is not universally true and some of the most intense reactions were observed

in tests of infant serum. (b) Isohemolysin "a" which occurs in serums of Groups III and IV is much more frequently present at birth than isohemolysin "b" which occurs in serums of Groups II and IV. This tendency for isohemolysin "a" to predominate is also seen in adult serums but to a much less degree than in infant serums.

3. When an excessive amount of red cells which have been twice washed in physiologic sodium chlorid solution is used for the isohemolytic tests, hemolysis will be inhibited. This inhibition of hemolysis seems to be due to the presence of antihemolysin in the cell suspension. Even after five washings in physiologic sodium chlorid solution some antihemolysin seems to be present in cell suspensions.

I wish to express my thanks to the members of the staff of the Boston Lying-In Hospital, whose assistance made possible the study of infant blood, and to Drs. George R. Minot, Roger I. Lee and William L. Moss for advice and criticism.

MODES OF INFECTION IN PYELITIS*

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Having spent several years studying various phases of the pyelitis problem, it has become very clear to me that a correlation of the clinical, pathologic and bacteriologic findings must be established. Strange as it may seem, this really has not been accomplished. A short survey of the difficulties in our way may prove profitable.

There is no correlation between the severity of symptoms and the pathologic findings. The former cannot be used at present as a means of localizing the infection. The same symptoms may be associated with cortical abscesses of the kidney, with infection of the pelvis, ureters or bladder, singly or combined. In spite of the severe clinical course there are, at best, only few postmortem examinations, in which the pelvis alone is found to be involved. Accordingly, it is impossible to make a diagnosis from the clinical picture alone, and if we had to rely on the lesions found at postmortem, pyelitis, uncomplicated by severe kidney lesions, would be extremely rare. The disease termed pyelitis, or pyelocystitis, or pyelonephritis, is very common. The symptomatology of infections of the urinary tract is so bizarre that the diagnosis of pyelitis or pyelocystitis, according to common opinion, rests entirely on urinary findings. It must be granted that the examination of the urine in most instances will indicate a pathologic process somewhere in the urinary tract, but that is all; it cannot localize the lesion.

I am omitting from the discussion all the cases that are associated with a damming back of the urine and the rupture of abscesses into the urinary tract, and shall consider only the group of cases in which the urinary tract is unobstructed. In order to consider the evidence under headings that will aid in an understanding of the problems, the material has been grouped.

1. Urinary cultures
   - Bladder
   - Ureter
   - Necropsy
     - Cortex
     - Pelvis
     - Bladder

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2. Pathologic anatomy
   Necropsy material
   Experimental material
3. Blood cultures
4. Experimental production of pyelitis
   By intravenous injection
   By intracystic injection

In a recent article Langer and Soldin [1] state that in a study of 138 patients they were able to isolate the *Streptococcus lacticus* in every instance in which repeated cultures were made. The cultures were made only in liquid mediums and are, therefore, open to criticism. In a former series of experiments, carried on by Beeler and Helmholz,[2] the urine was found to be sterile in about 50 per cent. of normal patients and in 30 per cent. of the patients with parenteral infection; solid mediums were used. In so far as no cultures were made in liquid mediums, and Langer and Soldin emphasize the importance of liquid mediums for the growth of the *Streptococcus lacticus,* it was determined to repeat these experiments. The urines from seventy patients (thirty-nine boys and thirty-five girls), were examined. In thirty cases (seventeen girls and thirteen boys), the urine was sterile in all mediums.

To summarize: In liquid mediums, cultures were sterile in fifty-nine of 108 instances, and in solid mediums in seventy-five of 108 instances. In only eight instances was it possible to identify the *Streptococcus lacticus.* This was done by its reaction in milk, as outlined by Hastings of the University of Wisconsin.

In only ten of 108 instances were there more than ten bacteria to the cubic centimeter in any of the specimens examined. Because of the difficulty of properly sterilizing the male and the female genitalia, it may be justly assumed that the gross, as well as the slight, contaminations may be attributed to errors in technic, or to the possibility that a certain number of bacteria may be carried into the bladder with the catheters.

So far as bladder cultures concern the mode of infection, they would be important only if Langer and Soldin's premises were correct, namely, that the organisms present in the specimens obtained from the bladder always come from the kidney. Inasmuch as it is impossible under any circumstances to prove this, cultures of the bladder cannot be of use in helping to solve the problem of the mode of infection, but are of importance only in detecting an infection in the urinary tract.

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1. Langer and Soldin: Zur Aetiologie der Säuglingspyelitis, Ztschr. f. Kinderh. **19**:161, 1919.

2. Beeler and Helmholz: Bacteriology of Urine, etc., Am. J. Dis. Child. **12**:345 (Oct.) 1916.

Large series of cases of pyelitis have not been studied, except by bladder cultures, and by this means all that can be determined is that the bacteria come from the urinary tract. It is acknowledged by all that it is practically impossible to differentiate, clinically, cystitis, pyelitis and acute multiple abscesses of the kidneys. By means of ureteral catheterization we have advanced a step in being able to determine whether the infection is in the bladder or higher. Hinman,[3] in his report of twelve cases, found that in six the bladder alone was involved. In the other six the pyelitis was unilateral in two cases and bilateral in four. In all six cases the bladder also was involved. Helmholz and Kretschmer,[4] in a series of eleven cases, found bilateral involvement of the kidneys and of the bladder. Hinman very rightly says that his finding of simple cystitis in one half of the cases speaks in favor of the ascending mode of infection as against the hematogenous. Ureteral catheterization affords very little help in determining the mode of infection in pyelitis, but if carried out in a sufficiently large group of cases of the different types, it shows the numerical distribution of infections of the bladder and kidney, and it may make possible a regrouping of cases, the features of which will differentiate cystitis from inflammation of the upper urinary tract.

Not only is the number of necropsies in cases of pyelitis relatively small, but in those performed there has been no satisfactory bacteriologic study. Moreover, it is only necropsies performed shortly after death that could be of any use in the study of pyelitis, since after death the colon bacillus rapidly penetrates into the various organs. In the literature there is practically no large series of cases that has been studied both bacteriologically and histologically. Inasmuch as it is impossible to diagnose clinically the involvement of a definite portion of the urinary tract, cultures made at necropsy could afford valuable information, but never more than to indicate the uppermost seat of involvement. In order to study the pathologic anatomy of pyelitis, it is absolutely essential that these cultures be made.

The importance of bacteriologic studies is demonstrated by our pathologic studies of kidneys. These tissues were used for making serial sections of the pelvis of the kidney. No cultures were made at necropsy of the different portions of the urinary tract. Clinically, the cases were definite examples of pyelitis; grossly and histologically, they showed, in the serial sections, an entirely normal pelvis. The results should have shown, therefore, that pyelitis may occur without a demonstrable inflammatory condition of the pelvis of the kidney at necropsy,

3. Hinman: Cystoscopic Study of Urologic Conditions in Young Children, Am. J. Dis. Child. **17**:305 (April) 1919.

4. Helmholz and Kretschmer: The Treatment of Pyelitis in Infancy and Childhood, J. A. M. A. **75**:1303 (Nov. 13) 1920.

but there is no definite reason why an inflammation of the pelvis should have been expected, since from the history and from the various examinations no definite localization within the urinary tract was possible. In other words, it was not certain that pyelitis was actually present. The pathologic report in so many cases of pyelitis is negative that it seems essential that bacteriologic studies should be made of the kidney pelvis and of the bladder in all cases of pyelitis that come to necropsy. These cases are so rare that only by making a group study of carefully controlled material will it be possible to reach any definite ideas with regard to the pathology. The problem is still further complicated by the fact that even at necropsy shortly after death the mucous membrane of the pelvis is found to be macerated by the urine, and specimens of urine withdrawn from the pelvis are practically always cloudy or purulent, but on microscopic examination show only a large number of desquamated epithelial cells indicating the rapidity with which the living mucous membrane is destroyed.

The pathologic findings of Thiemich [5] are most often cited in the discussion of the pathology of pyelitis. A bacteriologic study was not made in his series of cases. His patients had abscesses of the cortex of the kidney and, as he particularly points out, no lesions of the pelvis and the bladder. One girl and one boy had acute lesions with abscesses; two other patients had lesions of a more chronic nature, showing focal sclerosis in the cortex. It has been the experience of most authors that abscesses of the cortex of the kidney in man are not caused by colon bacilli and, in the absence of any bacteriologic studies, it is fair to assume that these conditions were due to infections by one of the coccus group. Such being the case they are not concerned in this discussion.

The "pyelitis" most frequently observed at necropsy is that with lesions of the kidney. In another type death is caused by some intercurrent infection. In these cases, as a rule, no lesions are found macroscopically or microscopically. Most of these were not controlled bacteriologically and are, therefore, of very little use. One point to be emphasized in the histologic studies of Thiemich is the fact that, in spite of the large amount of infectious material that must have passed through the pelvis in his four cases, there was no involvement of the mucous membrane of the pelvis. This is in answer to those who maintain that the colon bacillus is rapidly excreted through the kidney and thence gains a foothold in the pelvis, where we know that it remains only a very short time before it is carried on into the bladder. Only on the assumption that the colon bacillus finds in the

5. Thiemich, M.: Ueber die eitrigen Erkrankungen der Nieren und Harnwege im Säuglingsalter, Jahrb. f. Kinderh. **72**:243, 1920.

pelvis ideal conditions for its growth, is it justifiable to make an assertion, which no one has proved to be true. Furthermore, in those cases of renal involvement definitely secondary to lesions in the lower urinary tract, there is usually such a marked destruction of tissue anatomically, that the histology is of practically no use in helping us to solve the problem of the mode of infection. The majority of these cases are associated with some factor damming back the flow of urine.

I wish to come back for just one moment to our histologic study of the two cases of pyelitis by serial sections of the pelvis. I mention these principally as a warning to others of how not to make such studies, and to emphasize again the necessity of localizing the infection to the pelvis of the kidney before attempting to study it histologically. It is much simpler to study the pathologic anatomy of experimental pyelitis. The reported findings tend to indicate that the pathologic picture is somewhat different in the pelvis of the kidney when the infectious material reaches it by way of the blood stream than when it reaches it by the ascending route. The experimental work of Helmholz and Beeler [6] in the rabbit showed that cortical lesions occur in a large percentage of the animals following hematogenous injection and that the inflammatory condition in the pelvis tends to localize in the papilla. This has been recently confirmed by Lepper [7] who was able to show that bacilli tended to localize in the capillaries of the papilla and to produce changes therein. In cases of lesions in the kidney caused by intracystic injections the acute lesions in the pelvis are essentially in the parietal portion of the mucous membrane and the papilla is usually free. In only one instance were cortical lesions observed. At present this is the only feature which permits a histologic distinction of hematogenous pyelitis from an ascending pyelitis.

David and Matill,[8] in their experimental work, repeatedly found polymorphonuclear exudate in the submucosa of the pelvis, and sterile urine on the same side. They emphasize the importance of differentiating periureteral and peripelvic infiltration and an infection of the urinary tract. In all our experiments in which we found peripelvic inflammation, there was also found an infection of the urinary tract, as evidenced by the presence of bacteria and pus within the lumen of the pelvis.

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6. Helmholz and Beeler: Ascending Infection of the Urinary Tract, Tr. Am. Ped. Soc. **30**:196, 1918.

7. Lepper, E. H.: The Production of Coliform Infection in the Urinary Tract of Rabbits, J. Path. & Bacteriol. **24**:192.

8. David, Vernon C., and Matill, P. M.: The Rôle of the Ureteral Lymphatics in Experimental Urinary Tract Infections, Arch. Surg. **2**:153 (Jan.) 1921.

The fact that in two-thirds of the infections of the pelvis, after intracystic injections, no periureteral infiltration was found, but only parietal peripelvic inflammation and pus in the pelvis, shows that here, too, is an histologic differentiation of the mode by which infection may travel from the bladder to the kidney. Further studies with serial sections should do much to clear up the present uncertainty with regard to the pathologic anatomy of pyelitis.

In the experimental production of pyelitis, there is good evidence for the production of inflammatory conditions of the pelvis of the kidney, both by the hematogenous and the ascending routes. The hematogenous method is very frequently associated with abscesses in the cortex of the kidney, but also in numerous instances by a simple inflammatory condition of the pelvis. This has been accomplished by Bumpus and Meisser[9] with streptococci isolated from carious teeth and in cases of pyelitis, by Helmholz and Beeler,[10] with an organism isolated from a spontaneous case of pyelitis in the rabbit. A large number of observers, for example Eisendrath and Schultz,[11] and many others, have shown that with partial obstruction of the urinary tract, ascending infections can be readily produced. The work of David[12] in the production of pyelitis with the unobstructed urinary flow without any periureteral or peripelvic inflammation and with a sterile hydronephrosis on the opposite side, shows very definitely the production of pyelitis by ascension through the lumen of the ureter. Experiments of Helmholz and Beeler[6] in rabbits with a normal urinary tract showed definitely that infection in some instances travels by way of the periureteral and peripelvic lymphatics and, in some instances, directly by way of the ureteral lumen. This latter is in accord with the observations of Kretschmer[13] and others, that regurgitation of urine from the bladder into the ureter, under normal conditions, does occur. With an inflamed, thickened bladder wall around the ureteral valve, regurgitation can take place even more readily.

The presence of the colon bacillus in the blood stream has been used as one of the main reasons for assuming the hematogenous route of infection of the pelvis. Numerous observers, such as Kowitz,[14]

9. Bumpus & Meisser: Arch. Int. Med. **27**:326 (March) 1921.

10. Helmholz and Beeler: Experimental Pyelitis in the Rabbit, J. Urol. **2**: 395, 1918.

11. Eisendrath and Schultz: Pathology of Involvement in Ascending Infections, etc., J. M. Research **35**:337, 1917.

12. David, V. D.: Ascending Urinary Infections: Experimental Study. Surg. Gyn. & Obst. **26**:159 (Feb.) 1918.

13. Kretschmer: Cystography, Surg. Gyn. & Obst. **23**:709, 1916.

14. Kowitz: Ueber bakterielle Erkrankungen der Harnorgane im Säuglingsalter, Jahrb. f. Kindh. **32**:309, 1915.

Cabot and Crabtree,[15] Trumpp,[16] and others, have demonstrated the colon bacillus in the circulating blood. They consider this finding sufficient to accept the hematogenous route of infection of the kidney as a fact. Recently Lepper [7] has shown that experimental bacteriuria may be present for many days without any organism appearing in the urine. This is diametrically opposed to the frequently quoted experiments of Biedl and Kraus [17] which are open to criticism because of the possible injury to the kidney caused by the introduction of catheters into the ureters. The experiments of Cabot and Crabtree [15] are of particular interest because the blood cultures were positive when the urine was sterile and followed shortly after colon bacilli could be demonstrated in the urinary tract. There can be no question that pyelitis and cortical lesions of the kidney can be produced in the animal by the hematogenous route, by organisms of the coccus as well as of the colon group. The exact mode by which the organisms reach the pelvis and produce inflammation is still open to question, although in all likelihood it is not by way of the usual excretory channels but by way of the capillaries of the papilla. Various authors seem to think that the demonstration of the possibility of the hematogenous route of infection excludes any other route of infection.

With regard to the direct passage of organisms from the intestinal canal to the kidney by way of the lymphatics, it does not appear that there has been sufficient evidence brought forward to warrant a discussion at this time. In view of the manner in which the lymphatic system develops, there is a possibility of a direct connection persisting in some cases.

The clinical term pyelitis has been used to describe a great variety of pathologic conditions, all associated with the common symptom of pyuria. Clinically, it is impossible to differentiate the different forms involving the kidney cortex, pelvis, ureter, and bladder, singly or together. The pathologic anatomy of these various forms of pyelitis is not well established, and does not, at the present time, allow us to determine the mode of infection, except in those cases marked by cortical abscesses of the kidney. Only by a careful correlation of the findings obtained by bacteriologic and pathologic study, as well as by experimental work, can we hope to reach a better understanding of this problem.

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15. Cabot and Crabtree: Etiology and Pathology of Nontuberculous Renal Infections, Surg. Gyn. & Obst. **23**:495, 1916.

16. Trumpp: Cases of Cystitis in Boys and Girls, Jahrb. f. Kinderh. **5**: 268, 1897.

17. Biedl and Kraus: Ueber die Auscheidung der Microorganismen durch die hiere, Arch. f. Exper. Path. u. Pharmakol. **7**:1 1896.

STUDIES OF INFANT FEEDING. XV.

THE CALCIUM OF COW'S MILK IN ITS RELATION TO THE DIGESTION AND ABSORPTION OF CASEIN. PROTEIN CURDS IN STOOLS *

ALFRED W. BOSWORTH

BOSTON

It is quite generally held that the presence of hard protein curds in the stools from bottle fed infants is indicative of a faulty digestion of the casein fed in the modified cow's milk formulas.[1] Such a view seems to confuse the issue, placing on the baby a fault which should be borne by the person under whose direction the modified milk formulas are prepared, as Brennemann [2] has clearly demonstrated in four cases in which he was able to produce curds in infants' stools and make them disappear at will by manipulation of the food.

The chemical composition of these hard curds leads me to believe that they are not produced as the result of any fault in the digestive functioning of the infant but that they are produced by a normal digestion, functioning on an unnatural food which is only partly modified to a condition adaptable to the requirements of the infant.

Cow's milk, nature's food for the offspring of the cow, contains inorganic constituents in sufficient amounts to supply the demands of the rapid growth and development of this animal and is especially rich in calcium and phosphoric acid, substances which the young of the cow use to develop rapidly growing bone tissue, but which the infant uses very sparingly. When fed to infants in the proportions found in cow's milk, or modified cow's milk, these substances are greatly in excess of the amounts required to supply the demands of the infant, and hence are eliminated either in the urine or the feces.

In a previous paper [3] it has been shown that most modified milk formulas contain an excess of available calcium. In another paper [4] will be found data concerning the chemical compositions of the caseins from cow's milk and breast milk together with certain facts concerning the action of rennin upon these caseins. In this last paper it was shown that the nature of the curd produced by the action of rennin was dependent on two factors, the amount of soluble calcium and the

* Received for publication Aug. 15, 1921.

* From the Boston Floating Hospital Laboratories.

1. Courtney, A. M.: Am. J. Dis. Child. **3**:1 (Jan.) 1912.
2. Brennemann, J.: Am. J. Dis. Child. **1**:341 (April) 1911.
3. Bosworth, A. W.: Ann. Med. **1**: No. 4, 1921.
4. Bosworth, A. W.: Am. J. Dis. Child. **22**:193 (Aug.) 1921.

degree of acidity in the milk. In another paper [5] it was shown how the excess calcium present in cow's milk, when this milk is used as a food for infants, interferes with the assimilation of the fat.

In this paper are presented data concerning the relation between the calcium content of cow's milk and the digestion and absorption of casein by bottle fed infants.

THE CONDITION OF CALCIUM IN COW'S MILK

The calcium content of cow's milk may be separated by mechanical means into three fractions as indicated in Table 1.

TABLE 1.—CONDITION OF CALCIUM IN COW'S MILK

	Gm. per 100 C.c.
Calcium as insoluble phosphate ($CaHPO_4$)	0.051
Calcium combined with proteins	0.058
Calcium as soluble salts in serum (whey)	0.045

The insoluble calcium phosphate is inert in so far as its food value to the infant is concerned for it passes through the alimentary canal undigested and unabsorbed and appears in the feces. Its presence in milk, however, is a dangerous factor which must receive proper consideration, for, by the development of acidity within the milk, the calcium combined within the phosphate in an insoluble form is converted into soluble calcium. This soluble calcium, in addition to that naturally present in the milk, together with the developed acidity, have marked influence on the nature of the casein or paracasein curds formed by the action of the gastric enzymes.

Any increase in the amount of soluble calcium in milk also increases the possibility for the production of insoluble calcium soaps, which soaps may cause constipation.

If sanitary conditions are eliminated as a factor, the greatest danger arising from the use of cow's milk, which has been allowed to age, as a food for infants, rests in its content of insoluble dicalcium phosphate.

The other two forms of calcium, that combined with the casein and that present as soluble salts in the whey, are responsible for the loss of fat in the form of calcium soaps in the stools, as has been shown in a previous paper,[5] and in this paper we will show that they play an important part in causing the protein curds which are found in the stools from some bottle fed infants.

5. Bosworth, A. W., Bowditch, H. I., and Giblin, L. A.: Am. J. Dis. Child. **15**:397 (April) 1918.

THE CURDLING OF MILK AS INFLUENCED BY ITS CALCIUM PHOSPHATE CONTENT

Fresh cow's milk contains insoluble dicalcium phosphate which is present in colloidal form. This form of phosphate has practically no influence on the curdling of milk by rennin.

The development of acidity in cow's milk results in the production, from each two molecules of insoluble dicalcium phosphate, of two molecules of soluble calcium salts, i. e., one molecule of calcium lactate and one molecule of soluble monocalcium phosphate.[6]

$$2(CaHPO_4) + 2(C_3H_6O_3) = Ca(C_3H_5O_3)_2 + CaH_4(PO_4)_2$$

Insoluble dicalcium phosphate	Lactic acid	Soluble calcium lactate	Soluble monocalcium phosphate

The presence of these two soluble calcium salts, one of which, the monocalcium phosphate, is an acid salt, has a marked influence on the curdling of milk by rennin.

The effect produced by the development of acidity in milk by the common dairy organism has been shown in Table 5 of a previous paper.[4]

The effect produced by the addition of a mixture of the salts resulting from the development of acidity in milk will be found in Table 2.

TABLE 2.—SHOWING EFFECT ON THE CURDLING TIME OF MILK DUE TO THE ADDITION OF A SOLUTION CONTAINING EQUAL MOLECULAR CONCENTRATIONS OF CALCIUM LACTATE AND ACID CALCIUM PHOSPHATE

	Increase in Acidity Due to Salt Mixture Added	Acidity of 100 C.c. of Milk as N/10	C.c. Rennin Added	Curdling Time	
				Minutes	Seconds
1	0	10.5	1	61	50
2	1.5	12.0	1	10	5
3	3.0	13.5	1	5	45
4	4.5	15.0	1	3	45
5	6.0	16.5	1	3	00
6	7.5	18.0	1	2	15
7	9.0	19.5	1	1	50
8	10.5	21.0	1	1	40
9	12.0	22.5	1	1	25

This table clearly demonstrates the relation in the time required to curdle milk by rennin and if one should repeat the experiment he could not fail to note the increased toughness of curds and greater expression of whey produced by the addition of increased amounts of the salt mixture.

6. Van Slyke, L. L., and Bosworth, A. W.: J. Biol. Chem. **19**:23, 1914; **24**:191, 1916.

THE CONTROL OF THE NATURE OF THE CURD PRODUCED BY THE ACTION OF RENNIN IN MILK

In a previous paper [4] it has been shown that the curdling of milk by rennin is dependent upon the nature and amount of soluble bases present in the milk.

The curdling of the milk by rennin can be prevented or the consistency of the curd controlled by the addition to the milk of soluble salts of sodium, potassium or ammonium; the action of these salts being due to an interchange of the bases whereby some of the calcium combined with the casein is replaced by either sodium, potassium or ammonium as the case may be. This is shown in the following reaction:

$$\text{Casein } Ca_4 + K_2CO_3 \rightarrow \text{Casein } Ca_3K_2 + CaCO_3$$

In the above reaction two potassium atoms have replaced one calcium atom and in this case no curdling could be produced by the action of rennin.

The control of the curdling of milk by the addition of sodium carbonate is shown by the figures given in Table 3.

TABLE 3.—SHOWING THE INFLUENCE OF ADDED SODIUM CARBONATE UPON THE CURDLING OF MILK BY RENNIN

Number	C.c. 1.0% Na_2CO_3 per 100 C.c. Milk	Gm. Na_2CO_3 per 100 C.c Milk	Amount of Rennin Solution Added, C.c.	Coagulation Time, Minutes
1	0	0	2.5	0.5
2	0.5	0.005	2.5	0.75
3	1.0	0.01	2.5	2.0
4	1.5	0.015	2.5	3.25
5	2.0	0.020	2.5	3.50
6	2.5	0.025	2.5	6.50
7	3.0	0.030	2.5	8.00
8	3.5	0.035	2.5	11.50
9	4.0	0.040	2.5	13.00
10	4.5	0.045	2.5	14.50
11	5.0	0.050	2.5	29.00
12	5.5	0.055	2.5	38.00
13	6.0	0.060	2.5	50.00
14	6.5	0.065	2.5	No curd

In connection with the data given in this table it should be stated that a more concentrated extract of rennin was used than was the case in the preceding experiment in order to reduce the curdling time.

The curd formed in Sample 1 was a firm yet soft jelly-like mass. The other curds graded from this consistency toward very fine and soft flakes; in fact, it was rather difficult to measure the curdling time of the last few samples on account of the fine, soft condition of the curd.

A control of the curdling of milk may be obtained by the addition of lime water. The addition of lime water to milk makes it less acid and results in a decrease in the amount of soluble calcium due to the precipitation of insoluble calcium phosphate. If sufficient lime water

is added the soluble calcium can be reduced to such a small amount that the milk will not curdle upon the addition of rennin.

The nature of the curd formed by the action of rennin in milk may also be influenced by boiling the milk and by the action produced by the addition of such substances as malt soup, dextri-maltose No. 3 and Mellen's Food because they contain potassium salts, and barley gruel, etc., because of the physical action.

TABLE 4.—SHOWING THE INFLUENCE OF ADDED LIME WATER ON THE CURDLING OF MILK BY RENNIN

Number	C.c. of Lime Water per 100 C.c. Milk	Gm. $Ca(OH)_2$ per 100 C.c. Milk	Amount of Rennin Solution Added	Coagulation Time in Minutes
1	0	0	2	0.50
2	5	0.00925	2	3.33
3	10	0.01850	2	4.16
4	15	0.02775	2	5.50
5	20	0.03700	2	9.83
6	25	0.04625	2	15.00
7	30	0.05550	2	24.50
8	35	0.06475	2	81.66

RELATION BETWEEN THE CALCIUM IN THE FOOD AND THE NITROGEN IN THE FECES

Cow's milk is the food produced by nature for the nourishment of the calf and as the digestive organs of the calf are quite different from those of the infant it can digest the curds formed from cow's milk completely while the infant can not do so. Breast milk either does not curdle in the stomach of the infant or forms extremely fine soft curds.

TABLE 5.—SHOWING INFLUENCE OF CALCIUM IN THE FOOD ON THE AMOUNTS OF NITROGEN AND FAT ELIMINATED IN THE STOOLS OF INFANTS

	Kind of Food					
	Synthetic	Breat Milk	Synthetic	Synthetic	Modified Cow's Milk	Synthetic
Formula	3-6-1.5	3-6-1.5(?)	3-6-1.5	3-6-1.5	2-6-1.6	3-6-1.5
Calcium present as	None	Breast milk salts	$CaHPO_4$	Caseinate	Cow's milk salts	Caseinate and acetate
Casein present as	Sodium caseinate	Potassium caseinate (?)	Sodium caseinate	Calcium caseinate	Calcium caseinate	Calcium caseinate
Gm. CaO per 24 hours in food	0.00	0.40	0.45	0.51	1.13	1.55
Gm. CaO in feces per 24 hours	0.10	0.20	0.33	0.48	0.94	1.73
Gm. fat in feces per 24 hours	0.53	0.54	1.36	2.14	5.01	7.90
Gm. N in feces per 24 hours	0.16	0.20	0.22	0.41	0.54	0.73

The rate of digestion of the curds formed from milk depends upon the surface exposed. The large tough curds produced from cow's milk will be slowly and incompletely digested in the intestines of the infant, leaving a residue to be excreted in the feces while the fine soft curds produced from breast milk will be rapidly and completely digested.

We know that the removal of the soluble calcium from cow's milk prevents its curdling by the addition of rennin. If calcium is responsible for the curds formed from cow's milk and if the protein curds which appear in the stool are these same curds, undigested, we should expect to find some relation between the calcium and casein intake and the nitrogen eliminated in the stools. That such a relation exists is shown in Table 5.

The table distinctly shows the relation between the calcium in the food and the amount of nitrogen in the stools. As a matter of interest we have also given the figures for the amounts of fat (soaps and fats) eliminated. A relationship will also be noticed between the calcium intake and the fat excretion.

The so-called "synthetic milk" was made from butter fat, lactose, soluble casein, and the salts indicated in the table.

THE NATURE OF THE PROTEIN CURDS PRESENT IN THE STOOLS FROM SOME BOTTLE FED INFANTS RECEIVING MODIFIED COW'S MILK

A. M. Courtney [1] has given the analyses of many curds separated from infants stools. This work has been repeated by me and extended to include some determinations not made by Miss Courtney. The average results as found by these two sets of analyses are as follows:

TABLE 6.—AVERAGE ANALYSES OF PROTEIN CURDS FROM INFANTS' STOOLS

	Courtney, per Cent.	Bosworth, per Cent.
Protein	65	63.0
Fat	30	27.0
Ash	4	3.8
CaO	..	2.2
P_2O_5	..	1.3

These figures show that the curds are composed of protein, fat and ash and that the ash is almost wholly a mixture of calcium oxid and calcium phosphate.

To one familiar with the action of rennin in milk, and especially to one familiar with the chemistry of cheese making, these figures are very significant. When rennin is added to milk the curd formed is a mixture of fat, calcium paracaseinate and dicalcium phosphate. On standing these curds contract into tough masses.

In order to determine the nature of the protein in the curd the following procedure was carried out:

The dry curds were ground to a fine powder and extracted with ether to remove the fat. The protein residue was dissolved in a very weak solution of caustic soda and the protein precipitated by the addition of a little acetic acid. The precipitate was then treated by the

method of VanSlyke and Bosworth [7] for the preparation of pure casein and paracasein.

The final product was a fine white powder exactly resembling similar preparations of pure casein and paracasein previously made by the author.

It had a nitrogen content of 15.6 per cent. One gram required 2.50 per cent. calcium oxid to make a solution neutral to phenolphthalein. One gram required 2.25 c.c. tenth normal sodium hydroxid to form the soluble compound containing the smallest amount of base. These findings prove the substance to be paracasein.

CONCLUSIONS

1. The calcium and casein in cow's milk are the factors involved in the curdling of this milk by the action of rennin.

2. An increase in either the calcium content or the acidity of milk tends to toughen the curds produced by the action of rennin.

3. The calf has a digestive tract which can digest these curds. The digestive tract of the infant fails to completely digest these curds.

4. The inability of the infant to digest these curds does not indicate an impaired digestive functioning but does indicate a faulty supervision of the feeding of the infant.

5. The nature of the curds formed in cow's milk can be controlled by chemical and physical means.

6. The amount of nitrogen eliminated in the stools of infants may have a relation to the calcium content of the food.

7. The increase in the nitrogen content of stools from infants may be due to the presence of protein curds.

8. These protein curds are composed of protein, fat and dicalcium phosphate.

9. The protein in the curds is calcium paracasein.

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7. VanSlyke, L. L., and Bosworth, A. W.: J. Biol. Chem. **14**:203, 1913.

BOOK REVIEW

KOMPENDIUM DER KINDERHEILKUNDE MIT BESONDERER BERÜCKSICHTIGUNG DER SÄUGLINGSKRANKHEITEN. PROF. DR. ALBERT NIEMANN. Berlin, 1920. Verlag von S. Karger, Karlstrasse 15.

This compendium is of the usual type and comprehensiveness intended presumably for the undergraduate and the general practitioner. The range of discussion on any subject is always quite limited in a text of this kind. The author has very successfully brought out diagnostic essentials. Therapy receives rather scant attention. This is probably quite unavoidable but will prove disappointing to the large class of readers for whom such a book is intended. The subject matter deals particularly with diseases common to infancy and considerably less with those common to older children. The arrangement of the subject matter is quite different from that generally followed in textbooks. Etiological factors form the principal basis on which the division is made. On this basis there are roughly three large groupings: (1) Diseases due to congenital defects or influences; (2) diseases of the alimentary tract; (3) diseases due to infectious agencies. It was, of course, difficult with the complex etiology of many of the diseases to follow this division absolutely and there necessarily are some variations from it. In the main, however, the novel arrangement is successfully carried out. The chapters on constitutional anomalies and on feeding and alimentary disturbances are particularly good. The author's style is excellent and clear and concise. To anyone conversant with the German language the book should prove interesting and pleasant reading.

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